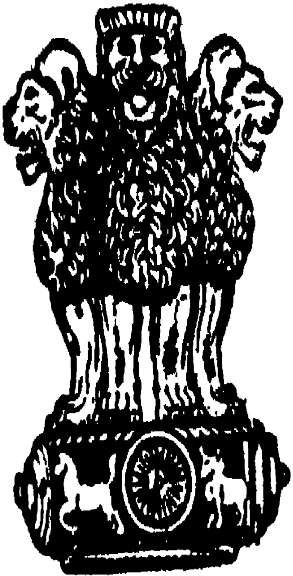

सत्यमेव जयते

INDIAN AGRICULTURAL
RESEARCH INSTITUTE, NEW DELHI.

47812

I. A. R. I. 6.

MGIPC—S1—6 AR/54—7-7-54—10,000.

Bulletin of the Agricultural Chemical Society of Japan.

Vol. 15.
(Nos. 172 - 183)
1 9 3 9

47812
IARI

The Agricultural Chemical Society of Japan.
c/o Faculty of Agriculture, Tokyo Imperial University.

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Bulletin of the Agricultural Chemical Society of Japan.

TRANSACTIONS

A New Bacterium (*Pseudomonas fibrolysis* n. sp.) Decomposing Cellulose.

By Yoshio OTANI.

From the Zymomycological Institute, Imperial College of Agriculture, Tottori, Japan.

(Received Dec. 9, 1938.)

The investigation concerning the bacteria decomposing cellulose has, hitherto, attracted the attention of many workers from various countries, and it is, now, one of the important problems in the bacteriological field. The decomposition of cellulose is generally carried on in nature aerobically or anaerobically, by a large number of different microorganisms. While making some experiments in this field, I isolated a new organism from a piece of decaying cloth, found in a pond. It developed and decomposed cellulose under aerobic conditions at ordinary temperature. I continued work with pure cultures, and I have described the morphology and other properties of this new organism, namely, *Pseudomonas fibrolysis*.

1. MORPHOLOGY.

The organism under observation appeared as small, slender, nonspore-forming rods. Branching or chain formation was not observed. It was Gram negative. In stained preparation from nutrient agar culture, after five days, the mean dimensions of the cells were found to lie in the region of 1.8 μ along the major axis; while the mean diameter is from 0.5～0.6 μ. Endospore was not observed. The organism is actively motile with side polar flagella.

2. CULTURAL CHARACTERISTICS.

These were studied on nutrient agar plates and slants, nutrient gelatin, and potato cylinder, as well as in nutrient broth, milk, litmus milk, and cellulose enrichment solution (K_2HPO_4 1 g, $MgSO_4 \cdot 7H_2O$ 1 g, NaCl 1 g; $CaCO_3$ 2 g, KNO_3 2 g, a piece of filter paper and water 1,000 cc). All cultures were incubated at 22°C to 28°C.

Nutrient agar plate: The colonies formed on this medium had various sizes, from 3 to 5 mm in diameter, after three days incubation. The surface colonies were round or roundish with well defined elevated edges, and faintly shining. Internal colonies were coarsely granular. A yellow colour was presented in some

ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

On the Method of Quantitative Estimation of Glutathione in Tissue by Okuda and Ogawa and its Modification by Fujita and Numata.

(Biochemical Studies on Glutathione. The Eighth Report.)

(pp. 1~7)

By Masayoshi OGAWA.

(Department of Nutrition, College of Medicine, Nippon University, Received Oct. 4, 1938.)

Several years ago (1933) Okuda and the author established a method of estimating reduced and oxidized glutathion in tissue and in biological fluids based on the same principle as that of so called Okuda's Iodine Method for the determination of cystein and cystin (1929). In this method, first of all, protein is removed by means of sulphosalycylic acid and the filtrate is divided into two portions. In one portion reduced glutathione is estimated directly by the titration at 0°C with $M/10{,}000$ KIO_3 solution, in the presence of certain quantities of iodid and starch. In the other, oxidized glutathione, after being reduced into reduced form by boiling the acid solution with zinc powder, is estimated in the same way. Subsequently (1938) the author corrected the iodate titration value for ascorbic acid and others by means of 2, 6-Dichlorphenol-indophenol treatment.

However (1938) Fujita and Numata reported a method for the determination of glutathione. Their method consists of titrating reduced glutathione in metaphosphoric acid filtrate with $N/1{,}000$ KIO_3 at 0°C in the presence of KI and starch and of reducting oxidized glutathione by means of H_2S in the acid slution for 12 hours at room temperature before titration, thus iodate titration value was corrected by means of ascorbic acid oxidase treatment.

Accordingly their method is entirely based upon the same principle as that of the above mentioned Okuda and the author's method determining GSH and GS·SG compound. The chief differences between their method and our's are as follows :— 1) According to their method the extraction is made with metaphosphoric acid. 2) The reduction is carried out by means of H_2S for 12 hours. 3) The results of iodate titration value are corrected with ascorbic acid oxidase treatment.

In the present communication, in order to ascertain the accuracy of their method the author made some experiments with the following results :—

Table I. Reduction of GS·SG into GSH by means of Zn powder and H_2S.

Glutathione found. (mg %)		Reduction by means of			
		Boiling with Zn dust for 20 minutes.		Saturating with H_2S for 12 hours.	
Sample	GSH	GS·SG	Total	GS·SG	Total
Liver	178	13	191	7	185
Heart	44	82	126	80	124
Lung	144	33	177	31	75
Kidney	130	50	180	46	176
Spleen	127	145	272	125	252

Table II. GSH value of yeast, which was corrected by means of 2, 6–Dichlorphenol-indophenol or ascorbic acid oxidase treatment.

GSH found (mg %)	GSH values: correction was made by means of	
	2, 6-Dichlorphenol-indophenol.	Ascorbic acid oxidase treatment.
I.	650	787
II.	680	735
III.	673	779
Means	668	767

From these above results the author concluded that reduction of GS·SG into GSH by means of H_2S treatment is nearly as effective as that of Zn dust, but GS·SG value of the former is slightly lower compared with that of the latter, and the reduction with H_2S treatment requires a longer time, while by means of Zn powder it ts rapid and more advantageous.

Iodate titration value (GSH) of tissue extract which was corrected by means of ascorbic acid oxidase treatment gives an extraordinary higher result compared with that of 2, 6-Dichlorphenol-indophenol in the case of dry beer yeast. Therefore, by means of ascorbic acid oxidase treatment the correction for iodate titration value of general tissue and biological fluids, with some exceptions, seems to be imcomplete which results in inaccurate estimation.

Okuda, Ogawa: The Journal of Biochemistry, Vol. XVIII, No. 1, 75, 1933.
Fujita, Numata: Tokyoijishinshi, No. 3093, 1938.

Statistic Studies of Soils. (IV)

(pp. 8～10)

By Dr. Misu-Hideo.

(Agricultural Experiment Station, Government General of Tyosen, Received June 24, 1938.)

On the Yeasts of the Genus *Schizosaccharomyces* indigenous to Iô-Island. (Ogasawara Islands)

(pp. 11～18)

Kin-ichirô SAKAGUCHI and Yosihisa ÔTANI.

(Tokyo Imperial University, Received Nov 15, 1938.)

The following 4 new varieties of Schizosaccharomyces pombe were isolated from the fermented molasses from Iô-island, one of the main island of the Ogasawara islands.

Schizosaccharomyces pombe var. *ogasawaraensis I.* nov. var.
Schizosacch. pombe var. *ogasawaraensis II.* nov. var.
Schizosacch. pombe var. *iotoensis I.* nov. var.
Schizosacch. pombe var. *iotoensis II.* nov. var.

Special studies were made on their particular enzymic activities, i. e. the *ureolysis, the liquefaction of gelatin and the fermentation of dextrins and inulin.*

Studies on the Enzymes of Lactic Acid Bacteria. (Part II).

On Lactic Dehydrogenase of Lactic Acid Bacteria.

(pp. 19～24)

By Kakuo KITAHARA.

(Department of Agriculture, Kyoto Imperial University, Received Nov. 10, 1938.)

It was found that *dl*-lactic acid was always dehydrogenated by all kinds of lactic acid bacteria under investigation in which resting cells of the bacteria were employed in the presence of methylene blue. However, bacterial cells were found to show complete optical specificities in oxidizing optically active lactic acids, as illustrated in the following table.

Species of Bacteria	Dehydrogenation of		
	d-Acid	*l*-Acid	*dl*-Acid
L. sake (*d*-former)	+	−	+
Leuconostoc (*l* former)	−	+	+
L. plantarum (*dl*-former)	+	+	+

The author proposed, therefore, that the so-called lactic dehydrogenase could be divided into *d*- and *l*-enzymes, and concluded that L. sake would contain only *d*-enzyme and Leuc. mesenteroides would, on the contrary, contain only *l*-enzyme.

When the bacterial cells were treated with acetone, L. plantarum was proved to contain only *l*-lactic dehydrogenase, so that the reason why the bacteria attacked both *d*- and *l*-acids was attributable to the presence of racemiase which had been inactivated by acetone.

For the dehydrogenated product of lactic acid, pyruvic acid was easily isolated in a yield of about 60%. But neither acetaldehyde nor acetic acid were obtained. This proves that carboxylase never existed in the lactic acid bacteria, whenever they revealed homo- or hetero-fermentations.

Untersuchungen über die Herstellungsmethode des Bagasse-Zellstoffes. (II)

Über dem Kochen der Bagassen mit Wasser unter Druck und den Bagasse-Zellstoff nach dem Natronverfahren.

(ss. 25∼32)

Von Syosuke TANAKA und Muneo KOORIYAMA.

(Department of Industry, Government Research Institute, Taiwan, Japan, Received Oct. 31, 1938.)

On the Alcohol-Manufacture from Jerusalem Artichoke. (Part VIII)

Influences of Storage on the Alcoholic Fermentation of Jerusalem Artichoke.

(pp. 33∼42)

By Toshinobu ASAI and Tetsuo YUKINOURA.

(Agricultural Chemical Laboratory, Morioka Agricultural College, Japan, Received Nor. 14, 1938.)

On the Koji-Amylase.

Part XII.—Studies on Some Protective Substances for the Heat Inactivation of α- and β-Amylases or Maltase, and on the Regeneration of these Enzymes being Inactivated by Heat.

(pp. 43∼49)

By Yuzo TOKUOKA.

(Tsunekichi Okura Brewery, Received Nov. 15, 1938.)

It was ascertained that Ca-ion revealed very remarkable protective action upon the heat inactivation of α-amylases, since in the presence of $CaCl_2$, the activity of α-amylase solutions of $pH=5.5\sim8.0$ (especially at pH $7.0\sim7.5$) was always found to be perfectly protected from heating at 40° for 20 hours, while 40∼100 % of its activity was lost by the same treatment without the addition of $CaCl_2$. This protective action was again revealed by the other Ca-salt as $CaHPO_4$, and very remarkable protection was found even in such a lower concentration of Ca-ion as $M/500$.

Any noticeable protective action was not observed with NH_4-, Na-, K- and

Mg-ions.

Although Ca-ion produced very much the same result with the preparations of α-mylases obtained from various materials including pancreatin, barley malt, Takadiastase and Saké-Koji, no evidence for the protective action of Ca ion was obtained with maltase of Saké-Koji or with β-amylase prepared from wheat and Saké-Koji.

Starch, protein and their decomposition products revealed protective action upon α-amylase in the order of $CaCl_2$ > starch > dextrin > peptone, egg-white > maltose > glucose, and very low degree of protection of maltase and of β-amylase was produced by these organic substances.

A part (nearly 5%) of α-amylase was regenerated within 2 hours by cooling to 25~27° from its inactive state produced by heating at 80° for 15 minutes.

More remarkable (nearly 70%) regeneration was obtained with maltase and β-amylase when these enzymes were inactivated by heating at 90° for 30 minutes.

Noticeable inhibition on the regeneration of inactive maltase and β-amylase was observed with the organic substances mentioned above.

Studien uber Sericin.

III. und IV. Mitteilung.

(ss. 50~55)

Von Takeo Ito und Kozo Komori.

(Aus dem chemischen Laboratorium der Seidenbau-Hochschule, Kyoto, Eingegangen am 8, 11, 1938.)

III. Nachdem der N-Gehalt von Sericin-A und Sericin-B festgestellt worden ist (Diese Zeitschr. 13, 115, 1937), haben wir durch "van Slyke's" Analysierung versucht derselben N-Verteilung zu bestimmen. Die erhaltenen Resultate sind in Tab. 1 zusammengestellt.

Tabelle 1. N-Verteilung von Sericin-A und Sericin-B.

	Ammoniak-N	Melanin-N	Zystin-N	Arginin-N	Lysin-N	Histidin N	Mono-amino-N	Nicht-amino-N	Summe
Sericin-A	12.14	0.17	0	9.15	4.70	2.71	70.10	0.61	99.58
Sericin-B	10.22	0.90	0	9.68	4.72	3.80	69.19	1.14	95.65

Es ist leicht ersichtlich, dass sich die beiden Sericinfraktionen dadurch am merklichsten voneinander unterscheiden, dass der Melanin-N-Gehalt vom Sericin-B, im Vergleich zu demjenigen vom Sericin-A, weit grösser ist.

Ferner wurden Untersuchungen bezüglich Gehalts an Kohlenhydrat und Amino-Kohlenhydrat der genannten Proteine durchgeführt.

Dabei wurde das Kohlenhydrat nach Tillmans und Philippi (Biochem. Z. 215, 36, 1929), und das Aminokohlenhydrat nach Nilsson (Biochem. Z. 285, 386, 1936) colorimetrisch bestimmt. Es ergab sich, wie aus Tab. 2 ersichtlich ist, dass die

beiden Kohlenhydratsorten in höherem Masse im schwerlöslichen Sericin-B, als im leichter löslichen Sericin-A, nachweisbar sind. Diese Tatsache wird mit den oben berichteten Resultaten über Melanin-N-Gehalt in engem Zusammenhang stehen.

Tabelle 2. Kohlenhydrat- und Aminokohlenhydrat-Gehalt von Sericin-A und Sericin-B.

	Kohlenhydrat als Glukose berechnet.	Aminokohlenhydrat als Glukosamin berechnet.
Sericin-A	0,45 %	0,37 %
Sericin-B	1,08 %	1,25 %

IV. Wir haben früher mitgeteilt, dass durch Behandeln der Kokonschicht mit heissem Wasser, Ammoniak entsteht. Um die Frage, ob dieses aus dem Sericin der Kokonschicht oder aus deren Fibroin entsteht, aufklären zu können, wurde die vorliegende Untersuchung angestellt.

0,2 g Sericin wurden mit 100 ccm Wasser in einem zweckmässig kons ruierten Apparat erhitzt, und dabei wurde das entstehende Ammoniak bestimmt. Folgende Tabelle gibt die Ergebnisse der Versuche wieder.

Tabelle 1. Ammoniak Abspaltung: mg Ammoniak-N pro 100 g Sericin.

Temperatur	100°		110°		120°		130°	
Erhitzungsdauer	Sericin		Sericin		Sericin		Sericin	
	A	B	A	B	A	B	A	B
1 Std.	—	—	150	98	—	—	—	—
2	122	69	231	155	394	304	666	505
4	220	141	414	262	662	444	1029	791
8			586	404				

Man sieht, dass sowohl Sericin-A als auch Sericin-B, wenn sie im Wasser erhitzt werden, Ammonia abgeben, und zwar das Erstere in höheren Masse als das Letstere.

Versuche mit solchem Fibroin, welches aus Kokonschicht durch vorsichtige Degummierung hergestellt wurden, folgendermassen ausgeführt: 2 g Fibroin wurden mit 100 ccm Wasser 2 Stunden lang bei einer gegebenen Temperatur erhitzt, dann nach dem Abkühlen filtriert und gut gewaschen. Filtrat und Waschwasser wurden vereinigt und analysiert. Mit dem zurückgebliebenen Fibroin wurde die gleiche Operation wiederholt. Tab. 2 gibt einen Teil der erhaltenen Resultate wieder.

Tabelle 2. Der bei der Heisswasser-Behandlung des Fibroins in Lösung gehende Gesamte- und gleichzeitig entstehende Ammoniak-Stickstoff in mg pro 100 g Fibroin. Die Erhitzung dauerte jedesmal 2 Stunden.

Temperatur	100°	110°	120°		130°
	Ammoniak-N	Ammoniak-N	Gesamte-N	Ammoniak-N	Ammoniak-N
Bei der 1 sten Behandlung	14	30	512	51	82
〃 〃 2 〃 〃		22	383	31	
〃 〃 3 〃 〃		13	383	23	
〃 〃 4 〃 〃		18	362	23	
〃 〃 5 〃 〃		13	353	22	
Summe		96	1993	150	

Tab. 2 lehrt, dass das Fibroin bei selbst wiederholtem Erhitzen im Wasser wenn auch nur in geringer Menge Ammoniak abgibt. Bei Berucksichtigung der Tatsache, dass die Kokonschicht hauptsachlich aus 20~25% Sericin und 75~80% Fibroin besteht, kann man aus den oben erwahnten Ergebnissen folgern, dass das bei der Heisswasser-Behandlung der Kokonschicht entstehende Ammoniak, grosstenteils aus Sericin, aber auch teilweise aus Fibroin stammt.

Es wurde ferner festgestellt, dass das Fibroin Kohlenhydrat enthalt, und zwar 2 mg (als Glukose berechnet) pro 100 mg Fibroin-N. Es konnte nachgewiesen werden, dass dieser Kohlenhydratgehalt dann abzunehmen beginnt, wenn das Fibroin in der angegebenen Weise wiederholt mit heissem Wasser behandelt worden ist. Ändererseits erkennt man aus Tab. 2, dass im Teil von etwa 10% des ursprünglich im Fibroin enthaltenen Stickstoffs nach 5 maligem Wiederholen der genannten Behandlung bei 120°, in Lösung gegangen ist. Das beweist, dass somit ein Teil des Fibroins samt dem Kohlenhydrat aufgelöst wird.

Ohara (Sc. Pap. I. P. C. R. Japan, 21, 104, 1933) hat durch eingehende Untersuchungen sehr wahrscheinlich gemacht, dass die beim Erhitzen im Wasser auftretende Beschadigung des Fibroins nicht auf die Veranderung dessen Krystallstruktur, sondern auf die des nichtkrystallinischen Teils zuruckzufuhren sei. Damit dürfen wir annehmen dass die oben erwahnten Verhaltnisse mit Ohara's Feststellungen in engem Zusammenhang stehen. Wenn also der amorphe Teil des Fibroins beim Erhitzen im Wasser teilweise in Losung gehen würde, wahrend die krystallinische Mizelle ungelost zuruckbliebe, so wurde der N-Gehclt des Fibroins im Verlaufe der genannten Behandlung geringermassen zunehmen, denn die krystallinische Mizelle des Fibroins soll nach Mark und Meyer u. a. nur aus kleinmolekularen Aminosauren wie Glykokoll und Alanin bestenen. Tatsachlich wurde gefunden, das dieser sich nach 5 maligem Wiederholen der Erhitzung des Fibroins im Wasser bei 120° von den anfanglichen 18,78% auf 18,95% steigert. Die Zunahme ist zwar geringmassig, dennoch analytisch unverkennbar.

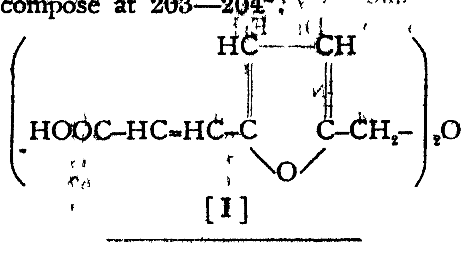
Synthesis of bis-[5-oxymethylfurfuracrylic acid]-ether.

(pp. 56).

By Kiyosi Aso.

(Tokyo Imperial University, Received Dec. 16, 1938)

The author synthesised bis-[5-oxymethylfurfuracrylic acid]-ether [I] from ω-oxy-methylfurfural-ether by means of Perkin's reaction. It crystallises in brilliant white plates which decompose at 203—204°.



[I]

Decomposition Products of Substance containing Uronic Acid in Autoclave (II).

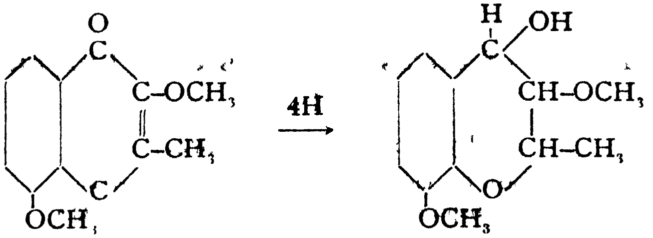
Reduction of Dimethyl-alginetin

(pp. 57~58)

By Kiyosi Aso.

(Tokyo Imperial University, Received Dec. 16, 1938)

The author obtained tetrahydro-dimethyl-alginetin (2-methyl-3, 8-dimethoxy-4-hydroxy-chroman) [II] by the catalytic reduction of dimethyl-alginetin (2-methyl-3, 8-dimethoxy-chromon) [I]. It forms silky needles, melts at 132°. The acethylderivative crystallises in colorless long prisms, melts at 117°.



[I] [II]

Synthesis of 3, 8-dimethoxy-flavone.

(pp. 59~60)

By Kiyosi Aso.

(Tokyo Imperial University, Received Dec 16, 1938.)

The author synthesised 3, 8-dimethoxy-flavone by heating 2-hydroxy-3, ω-dimethoxy-acetophenone (m.p. 66°) with benzoic anhydride and anhydrous sodium benzoate at 160~163° for 3 hrs.. It crystallises in yellow prisms, melts at 156~157°. Its two maximum of absorption spectrum are at frequency ca 3,320 and 3,920.

Chemical Studies on the Kikyô-root (Platycodon grandiflorum, A. Dc.) (Report IV)

On the Haemolytic Action and Toxic Properties of the Kikyô-root

(pp. 61~70)

By Magosaburo TSUJIMOTO.

(Kagoshima Imperial College of Agriculture & Forestry, Received Dec. 8, 1938.)

The object of my experiments was to determine the medicinal value of the kikyô-roots which differ in region, age, variety and preparation. For this purpose the haemolytic actions and poisonous properties of these samples were determined.

SUMMARY

1) Wild plants have more active haemolytic power than the cultivated ones.

2) Unrinded roots have more active haemolytic power than the rinded ones.

3) The intensity of haemolytic power increases according to the age of the plant. Thus: the second- > the first- > the third year plant.

4) Violet-flowering plants have more active haemolytic power than the white-flowering ones.

5) The poisonous properties*) of the kikyô-root run parallel with its haemolytic power.

Influence of Ultrashort-waves upon Enzymes.

(pp. 71~79)

By Yoshihisa TOGASAWA.

(Agricultural Chemical Laboratorv, Faculty of Agriculture, Hokkaido Imperial University, Received Dec. 8, 1338.)

The author intended to determine the effects of the specific action of ultrashort-waves upon enzymes, avoiding the heat effect caused by electric current.

Two, 5 and 10 m of ultrashort-waves (1,000~1,500 V) was not effective under various conditions on a number of commercial preparations (diastase, papayotin and pancreaslipase). The circuit used in this experiment was the Hertley circuit.

Biochemical Investigation of Mosaic Disease of Tobacco Plants. V.

On the Ascorbic Acid Content in the Leaves of Healthy and Mosaic Plants.

(pp. 80~86)

By Y. OKUDA and K. KATAI.

(Department of Agriculture of Kyushu Imperial University, Fukuoka; Received Dec. 12, 1938.)

On the Chemical Regeneration of Waste Tussah Silk. (Part I.)

Dispersion of Tussah Silk in Concentrated Solutions of Neutral Salts.

(pp. 87~92)

By Chiyoka MIYAHARA.

(Central Laboratory, South Manchuria Railway Co., Dairen. Received, Nov. 28, 1938.)

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*) The experiments were made on fishes.

Tussah silk is the silk secreted by the wild silk-worm, *Antheraea pernyi*, which is widely raised in Manchuria. The yield of waste silk produced by the present reeling process amounts to as much as 40% of the total silk layer of its cocoon. Although many studies have been made on the regeneration of waste Bombyx silk, very few investigations have been reported on those of Tussah silk.

The author has made fundamental studies on the dispersion of waste Tussah silk in concentrated solutions of $ZnCl_2$ and $Ca(CNS)_2$ with the object of its regeneration in mind. The results obtained are as follows :–

1. The dispersion media have the lower limits of concentration under which both Tussah and Bombyx silks are dispersible. With $ZnCl_2$ solution the lower limits of concentration are shown to be about 65% for Tussah silk and about 50% for Bombyx silk, while with $Ca(CNS)_2$ the lowest concentrations are about 50% for Tussah silk and about 30% for Bombyx silk, respectively.

2. The dispersibility of Tussah silk increases with the elevation of temperature as well as with the prolongation of reaction time up to 50∼60 hrs, when it reaches a maximum.

3. The refined Tussah silk is more easily dispersed than the raw silk, and the so-called "bleached silk" which is produced by the new reeling process using Na_2O_2 as a bleaching agent is more readily dispersible than the so-called "gray silk" which is made by the old plate reeling method.

On the Chemical Regeneration of Waste Tussah Silk. (Part II.)

Viscosity of Tussah Silk Solutions

(pp. 93∼98)

By Chiyoka MIYAHARA.

(Central Laboratory, South Manchuria Reilway Co., Dairen.. Received Nov. 28, 1938.)

The author has made studies on the viscosity of Tussah silk solutions when a concentrated solution of $Ca(CNS)_2$ was used as a dispersion medium with the following results :

1. The viscosity is greatly influenced by higher concentrations (13∼15%). The rate of increase of viscosity is much more remarkable than that at lower concentrations. The viscosity-concentration constant of dispersed Tussah silk is much smaller than that of Bombyx silk.

2. The viscosity decreases according to the elevation of dispersion temperature.

3. The viscosity decreases for a few hours after the dispersion, but increases gradually afterwards. After 1∼2 days it reaches a maximum, and then decreases slowly again.

4. Various substances such as phenol, α-naphthol, urea, glucose, sucrose,

glycerine, glycine and *l*-leucine added to the dispersion system somewhat increase its viscosity.

The Change in the Starch and Sugar Contents of the Tobacco Leaves after Topping.

(pp. 99～103)

By T. Nito and M. Ota.

(Government Monopoly Bureau, Japan, Received Oct. 28, 1938.)

Statistic Studies of Soils. (V)

(pp. 104～106)

By Dr. Misu-Hideo.

(Agricultural Experiment Station, Government General of Tyosen Received June 24, 1938.)

Partial Oxydation of Starch by Bromine.

(pp. ～)

By Yoshijiro Kihara.

(Tokyo Imperial University, Received Dec. 17, 1938.)

Starch paste was oxidized by dropping Br in it in the presence of $CaCO_3$ at room temperature. The excess of Br was removed from the reaction product by vacuum distillation, the product was filtered and a small amount of alcohol was added to the filtrate, yielding white precipitate. It showed uronic acid reaction by naphthoresorcin, and was named "Urondextrin". It was soluble in water, gave an acidic reaction and its rotation in aquecus solution was $[\alpha]_D^{15°}+181.72°$. It was precipitated by $Ca(OH)_2$ or $Ba(OH)_2$, but not by $CuSO_4$ nor Fehling's solution. Acetyl derivative, m p. 145°, was prepared, which also showed uronic acid reaction.

Urondextrin wae scarcely hydrolyzed by Takadiastase.

Vol. 15 February 1939 No. 2

Bulletin of the Agricultural Chemical Society of Japan.

ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

The Distribution of α-Sorinin and α-Sorigenin.

(pp. 109～111)

By Zirô NIKUNI.

(Agr. Chem. Laboratory, Tokyo Imp. Univ. Received Jan. 17, 1939.)

The author discovered a new glycoside from the bark of Kuroumemodoki and named it "α-sorinin" (Bulletin of Agr. Chem. Soc. of Japan: **14**, 25～26, 1938). This glycoside consists of primverose and an aglycon "α-sorigenin".

The aglycon α-sorigenin exists not only as a component of the glycoside but also in a free state. It was proved as follows:

The fresh bast fibre of Kuroumemodoki was boiled with 90～95% alcohol. On evaporation of alcohol yellowish fine crystals were precipitated. Dried crystals were extracted repeatedly with absolute alcohol. Upon this the glycoside remains insoluble. The yellowish brown absolute alcoholic solution was evaporated to small quantity and left overnight. Fine yellowish needles were crystallized out. These crystals dissolve in organic solvents, turn dark green by ferric chloride solution and become brown by the sunlight. This is the crude α-sorigenin.

The crude α-sorigenin was purified as diacetyl-α-sorigenin, and recovered from it as pure α-sorigenin.

3 g of the crude crystals and the same amount of Na-acetate were boiled with 60 cc of acetic acid anhydride and poured into 800 cc of ice water. The crude acetyl compounds thus obtained were recrystallized from glacial acetic acid and then twice from acetic ether. Almost colourless fine needles were obtained. M. p. 257～258.5°. No depression of melting point was observed when mixed with pure diacetyl α-sorigenin.

0.3 g of this diacetyl-α-sorigenin was warmed for 3 hours on water bath with 100 g of 2.5% sulphuric acid alcoholic solution. Thus the colourless needles were obtained. M. p. 225°. Mixed melting point with pure α-sorigenin was also 225°.

The analytical results of these compounds were as follows:

Diacetyl-α-sorigenin	C%	H%	OCH_3%
observed	62.36	4.76	8.57
calculated as $C_{13}H_8O_5(CH_3CO)_2$	61.79	4.27	9.39

α-sorigenin	C%	H%
observed (air dry)	58.85	4.63
calculated as $C_{13}H_{10}O_5 \cdot H_2O$	59.09	4.54
observed (dried at 100°)	62.82	4.14
calculated as $C_{13}H_{10}O_5$	63.39	4.09

Then the amounts of α-sorinin and α-sorigenin contained in the trees from different districts at different seasons were examined.

The trees were the following 3 species.

(1) Kuroumemodoki (Rhamnus japonica, Max. var. genuina, Max.)
(2) Kobano-kuroumemodoki (R. japonica, Max. var. decipiens, Max.)
(3) Kurotubara (R. dahurica, Pall. var. nipponica, Makino)

Species of tree	Growing district	Season	Bast fibre	Crude α-sorinin	Crude α-sorigenin
(1), (2)	Titibu	Autumn	1240 g	20.1 g (1.62%)	33 g (2.66%)
〃 〃	〃	〃	3500 g	55 g (1.57%)	28 g (0.80%)
〃 〃	〃	Spring	3770 g	35 g (0.94%)	45.5 g (1.18%)
(2)	Tiba	Winter	1050 g	3.8 g (0.36%)	21.8 g (2.07%)
(1)	Aomori	Spring	4190 g	12.7 g (0.30%)	14.2 g (0.33%)
(1)	〃	Autumn	5320 g	18.1 g (0.34%)	24.5 g (0.46%)
(3)	Morioka	〃	1070 g	——	——

The glycoside α-sorinin exists only in Rhamnus japonica, and the amount is most abundant in the trees from the Titibu Experimental Plantation of Tokyo Imp. Univ., especially in autumn.

The author expresses his sincere thanks to Prof. B. Suzuki for his kind guidance throughout this work.

Statistic Studies of Soils.

(pp. 112~114)

By Dr. MISU-Hideo.

(Agricultural Experiment Station, Government General of Tyosen, Received June 24, 1638.)

On the Manufacture of Alcohol from Jerusalem Artichoke. (Part IX.)

(pp. 115~127)

By Toshinobu ASAI and Susumu KIKUCHI.

(Agricultural Chemical Laboratory, Morioka Agricultural College, Japan,
Received Nov. 14, 1938.)

A New Alkaloid in the Bulbs of Narcissum Tazetta. L.

(pp. 128～132)

By Yoshijiro KIHARA.

(Tokyo Imperial University Received Dec. 17, 1938.)

Lycorin and tazettin have been found in the bulbs of Narcissus Tazetta L. Now a new alkaloid was isolated from N. Tazetta, by extracting the bulb with alcohol. The alcohol solution, which showed a remarkable violet fluorescence, was extracted with H_2O. The base was purified by precipitating with phosphotungstic acid, the precipitate decomposed with $Ba(OH)_2$, and the solution evaporated in vacuum into a syrup. When one drop of water was added, the syrup changed into a yellow mass. A light yellow needle was obtained by recrystallization from alcohol. It was named "suisenin". It melted at 229°C, and was given the formula $C_{17}H_{19}O_5N$. One CH_3O group, one OH group and one methylene-oxide group were proved in the molecule, but the presence of CH_3N group could not be observed.

Monomethyl-ester, m.p. 188°C, benzoylester, m.p. 196°C, hydrochloride, m.p. 180°C, picrate, m.p. 189°C and Pt salt, m.p. 194°C, were prepared.

From the absorption spectrum and the chemical reactions the presence of isoquinoline nucleus was presumed.

A crystal was obtained when suisenin was oxidized with $KMnO_4$ in alkaline solution, and this crystal melted at 244°C, the formula $C_{12}H_{15}O_4N$ was given to it.

Suisenin may be an alkaloid having phenanthridin nucleus like lycorin or tazettin.

The Change of Oxidation-reduction Potentials of Water-logged Soils.

V. On the application of the green manures in different stages of growth.

(pp. 133～138)

By Kisaburo SHIBUYA, Hideaki SAEKI and Kenhan RYU.

(Taihoku Imperial University. Received Dec. 19, 1938.)

On this occasion, the change of oxidation-reduction potentials was determined of water-logged soils, as these were mixed with green manures which were in different stages of growth. The soils taken for this investigation were a lateritic loam of Heichin and a clay derived from sandstone and shale of the University farm. Peas (Pisum sativum Linn.) and Sesbania (Sesbania Sesban (Linn.) Merrill) in young growing stages or sufficiently mature, were used as the green manures to be applied to the soils. The younger crops were more nitrogenous, while the matured plants were less so, being 8.7 or 11.2～11.8 of C/N quotient, respectively, from the analytical view point. This investigation should be compared with the authors' previous information (Jour. Agr. Ch. Soc. of Japan, XIV, 35, 1938.) which reported the potential change of water-logged soils applied with protein or carbohydrate. After thoroughly mixing with pulp of the green manures, the soils in a certain number of glass cylinders were kept at 30°C under the water-logged condition and the potentials were determined every day at the beginning and every week at the later period of the experiment which was continued for 10 weeks.

In consequence, the younger or more nitrogenous plants reduced the soil potentials, while the matured or large C/N materials lowered the potentials to less extent and slightly raised them at the later period.

Doubtlessly, the green manures make the soil condition more or less reductive, according to the younger or the matured stages of their growth when they are applied into the soils

Effects of Certain Minerals on the Growth of Root Nodule Bacteria. (Part 1)

(pp. 139~146)

By K. Konishi, T. Tsuge and A. Kawamura.

(Institute of Agr. Chem., Imp. University, Kyoto. Received Jan. 17, 1939.)

Spectrographical tests revealed that P and Mg contents of soybean and lupine bacteria grown on yeast water mannitol were higher but Na content lower than those of the other organism tested, and that, so far as P content is concerned, data on quantitative procedure also emphasized the above suggestion.

Phosphoric acid was usually effective in stimulating the growth of the organism, while large amounts of lime present in culture media were unfavourable for the development of either soybean or lupine bacteria. Antagonism between lime and phosphorie acid was recognized in affecting the growth of clover and soybean organism.

Chemical Studies on the Kikyo-root. (Report V)

The method for the separation and purification of Kikyo-Saponin, (Platycodin), and its properties.

(pp. 147~150)

By Magosaburo Tsujimoto.

(Kagoshima Imperial College of Agriculture and Forestry. Received Jan. 5, 1939.)

On the Determination of Exchangeable Lime in Calcareous Soil.

(pp. 151~155)

By R. Kawashima.

(Agr. Chem. Laboratory, Kyushu Imperial University. Received Jan. 10, 1939.)

In this paper the determination of exchangeable lime in soils containing calcium carbonate in precipitated form is dealt with. The author has confirmed that in such cases both Hissink's and Chapman & Kelley's methods are quite inadequate; while Puri's ammonium carbonate solution method is suitable.

Studies on the Vegetable Tannins in Formosa.

(pp. 156～160)

By. Yasuyosi Osima and Yôsuke Kaneko.

(Agr. Chem. Dep., Taihoku Imp. Univ., Received Jan. 16, 1939.)

The tannin contents were determined by the international official method of the Society of Leather Trade Chemists, the chemical properties of tannins were studied qualitatively by the method of Stiasny and others, and the tannins were classified according to Stenhouse-Procter's system.

The results were as follows:

Plant names	Water %	Soluble matter %	Non-tannin %	Tannin %	Classification
Bark of					
Acacia confusa Merrill	13.3	22.3	11.1	11.2	catechol
Trema orientalis Blume	13.2	24.4	17.3	7.1	″
Rhizophora mucronata Lam.	21.7	26.0	8.6	17.4	″
Psidium Guajava Linn.	9.1	30.2	16.7	13.5	pyrogallol
Albizzia falcata Backer ex Merrill	13.3	22.8	9.8	13.0	catechol
Uncaria formosana Hayata	21.7	11.3	5.7	5.6	″
Uncaria selilosa Bentham	8.9	24.4	15.1	9.3	″
Leaves of					
Uncaria setilosa Bentham	11.9	25.3	18.3	7.0	″
Psidium Guajava Linn	9.4	20.1	12.3	7.8	pyrogallol
Lagerstroemia subcostata Kochne var. Hirtella Kochne	11.5	32.5	19.2	13.3	″
Thea sinensis Linnaeus	10.4	40.1	26.0	14.1	catechol
Seeds of					
Areca catechu Linnaeus	11.5	18.2	5.3	12.9	″
Tubers of					
Dioscorea Matsudai Hayata	48.2	5.4	3.3	2.1	″
Dioscorea cirrhosa Loureiro	79.6	8.7	3.1	5.6	″

Decomposition Products of Substances Containing Uronic Acid. (III).

Reductic Acid.

(pp. 161～170)

By Kiyosi Aso.

(Tokyo Imperial University. Received Jan. 19, 1939.)

Saccharification of Starch with Organic Acids.

(pp. 171~176)

By Teijiro YABUTA and Kiyoshi ASO.
(Tokyo Imperial University. Received Jan. 17, 1939.)

Chemical Studies on Japanese Coccidae. (XXI).

Unsaturated Fatty Acids of *Tachardina theae* Green et Mann.

(pp. 177~181)

By M. KAWANO and R. MARUYAMA.
(Laboratory of Ohsaka Factory of Sankyo Co. Ltd., Received Dec. 20, 1938.)

The Effect of Glutathione on Fever.

Biochemical Studies on Glutathione. The IXth Report.

(pp. 182~192)

By Masayoshi OGAWA.
(Department of Nutrition, College of Medicine, Nippon University,
Received Dec. 26, 1938)

In the previous communication the author reported the glutathione contents of yeast preparations.

In the present report, he performed an experiment on the effect of glutathione (GSH, GS-SG), and cystein-HCl on fever, employing several rabbits which became feverish on account of intravenous glucose injections. Their temperature began to rise 2~3 hours after the injection reaching its maximum (not less than 40°C), after which it subsided to the normal level during the next 3~4 hours, while in the animals to which glutathione (GSH, GS-SG) or cystein-HCl 0.5~5.0 mg per kg of body weight was administered intravenously, fever abated within the first 2.2/3~3.1/2 hours; although it seemed to require about 4 times as much glutathione when it was administered under the skin.

From the above results the author concluded that: (i) the body temperature of the feverish animal subsided to the normal level by the action of the administration of glutathione or cystein-HCl. (ii) The average glutathione (GSH, GS-SH) requirement for the removal of fever seems to be about 0.5 mg per kg of body weight when administered intravenously, (iii) although in the case of hypodermic injection it seems to be about 2.0 mg per kg of body weight.

Blätteralkohol. III. Mitteilung.(1)

Riechstoffe der Gurken (Cucumis sativus, L.).

(pp. 193~195)

Von Sankiti TAKEI u. Minoru ÔNO.
(Aus d. Agriculturchem. Laborat. d. Universität Kyoto. Eingegangen am 28. Dez. 1938)

Da das aus Blatteralkohol (3-trans-Hexenol-1) synthetisch gewonnene 2-trans-6-trans-Nonadienal-1 (Veilchenblatteraldehyd)⁽¹⁾ einen an den Saft frischer Gurken erinnernden Geruch besitzt, vermuteten wir, dass voraussichtlich auch die Gurken ebendiese Substanz als Geruchsbestandteil enthalten würden.

Wir nahmen eine dementsprechende Untersuchung vor. Aus 144·7 kg frischer Gurken wurden durch Wasserdampfdestillation rund 2.5 g Rohöl gewonnen. Von diesem blieb nach der Reinigung mit Alkali und Säure 1.5 g stärk nach Gurkensaft riechendes Öles zurück. Bei der Fraktionierung dieses Öles wurde ein geringer Teil (ca. 10%) bis auf 80°/4 mm und ein grösserer Teil (ca. 60%) bis auf 100°/4 mm abdestilliert. Der Destillationsrückstand (ca. 30%) war geruchslos. Die erste Fraktion besteht aus 2-trans-6-trans-Nonadienal-1 (Semicarbazon, Schmp. 157° ; 2.4-Dinitrophenylhydrazon, Schmp. 113°), die zweite aber enthalt hauptsachlich 2-trans-6-trans-Nonadienol-1 (4′-Joddiphenylurethan, Schmp. 137° ; Allophanat, Schmp. 140°).

Hieraus also geht klar hervor, dass *bei den Riechstoffen der Gurken, der Qualität nach, 2-trans-6-trans-Nonadienal-1 vorherrscht, der Quantität nach aber, in der Hauptsache der diesen Aldehyd entsprechende Alkohol in Frage kommt.* Somit möchten wir diesen früher synthetisch erzeugten Alkohol, 2-trans-6-trans-Nonadienol-1, in praktischer Weise als "*Gurkenalkohol*" bezeichnen.

LITERATUR.

(1) II. Mitteilung: J. Agr. Chem Soc., 14, 717 (1938).

On the Stimulant for Cane Sugar Formation in Plants. (I).

(pp. 196～198)

By Tetutaro TADOKORO.

(Hokkaido Imperial University. Received Dec. 19, 1938.)

Chemical Studies on the Kikyo-root. (Report VI.)

Colloid Chemical Studies on Platycodin (Kikyo Saponin).

(pp. 199～205)

By M. TSUJIMOTO, R. SENJU and T. MATSUMOTO.

(Agr. Chem. Laboratory, Kyushu Imp. Univ. and Agr. Chem. Laboratory, Kagoshima Imp. College of Agr. and Forestry.)

SUMMARY.

The following colloid chemical properties of platycodin were examined.

1. Specific surface tension. (Table I)
2. Specific viscosity. (Table II)
3. Intensity of adsorption on animal charcoal.
4. Gold number.

5. **Cataphoresis.**
6. **Liesegang's phenomena.**

The experimental results were as follows:

Table I.

Concentration %	Water	0.01	0.02	0.04	0.06	0.08	0.12	0.14	0.16
Specific surf. tension	100.00	99.38	98.69	95.22	93.33	88.90	83.32	81.01	78.83

Table II.

Concentration %	Water	0.010	0.020	0.038	0.055	0.072	0.090	0.110	0.127	0.146	0.166
Specific viscosity	1.000	1.012	1.093	1.213	1.376	1.527	1.674	1.815	1.937	2.131	2.239

The empirical formula of adsorption is $\frac{x}{m}=0.102C^{0.518}$

The gold number is 16.

Platycodin is a negative colloid.

Liesegang's phenomena are evident.

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Bulletin of the Agricultural Chemical Society of Japan.

TRANSACTIONS

Studies on Vitamin B_2 Complex.

II—Some Observations on the Nutritive Value of Sugars.

By Ume TANGE and Tetsuko KANEKO.

(From the Institute of Physical and Chemical Reserch)

Received Oct. 23, 1938

Previously one(1) of us (U. T.) demonstrated that when rats were fed on sucrose diet they developed the pellagra-like symptoms and failed to grow, but this condition was protected and cured by acid clay adsorbate or its eluate of rice polishings extracts, and described that the factor might be identical with vitamin B_6 described by György.

Birch, György and Harris(2) showed that cane molasses is very rich in vitamin B_6. Furthermore, Jukes and Lepkovsky(3) recently found in cane molasses a fairly good source of the preventive factor, the so called filtrate factor, for chick dermatitis.

In nutrition as well as in domestic economy molasses is, therefore, more desirable to be used in daily life than pure white sugar, though molasses is still generally regarded as a qualitatively inert food constituent. It is very interesting to note that in ancient times "Kurozato" (a crude brown coloured sugar containing molasses) was commonly used by Japanese, whereas pure white sugar was used for medicine or only by rich people as precious food, and also that polished rice had been substituted for half-polished rice or "Haigamai" (rice with germ) which is more nutritious than the former.

The present paper contains the results of experiments on the nutritive value of "Kurozato", "Ganmitsuto" (a brown coloured sugar containing molasses), exhausted cane molasses, and sucrose.

EXPERIMENTAL.

Part I. (*Preliminary experiment*).

The methods employed in the present work were similar to those described in the previous paper.(1) When the weight of rats had remained stationary constant for several days, crystalline vitamin B_1 hydrochloride was supplied daily at the level of 10 γ.

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* This paper was presented at the Scientific Meeting of the Institute of Physical and Chemical Research, December 16, 1937.

The basal rations had the following composition: †

Component \ Rations	Sucrose	"Kurozato"	"Ganmitsuto"	Cane molasses
Purified fish protein	20	20	20	20
Crisco	3	3	3	3
McCollum's salt mixture	5	5	5	5
Dried egg white powder (as flavin)	5	5	5	5
Agar-agar	2	2	2	2
Sucrose (*pharmacopœia Japonica*)	65	—	—	—
"Kurozato" (commercial)	—	65	—	—
*"Ganmitsuto"	50	—	15	—
*Exhausted cane molasses	60	—	—	5

† In addition to the diets all animals received 1 drop of cod liver oil daily for vitamins A and D.

* "Ganmitsuto" and molasses were provided by Meiji-Seito Company. We wish to thank them for the generous contribution.

Analysis of Samples (%).

	"Ganmitsuto"	"Kurozato"	Exhausted cane molasses
Moisture	1.94	7.16	22.82
Solid	98.06	92.84	77.18
Sucrose	86.83	79.72	34.79
Invertose	3.22	8.00	17.72
Ash	5.94	3.59	14.13
Total nitrogen	0.03	0.14	0.42
Organic matter	2.04	1 39	10.12
	100.00	100 00	100.00

Components of Ash.

	"Ganmitsuto"	"Kurozato"	Exhausted cane molasses
Fe_2O_3	12.75	8.60	2.64
CaO	27.30	25.08	23.98
K_2O	36.17	22.80	45.25
P_2O_5	1.35	7.75	5.20
Insoluble portion	5.93	27.24	18.16
Remainder	16.50	8.53	4 77
	100,00	100.00	100.00

The results are summarized as follows:

1. The rats fed on the diet composed of 65 per cent sucrose as source of carbohydrate, supplemented daily with 10 γ vitamin B_1, developed pellagra-like symptoms and died within 6 to 8 weeks, while on "Kurozato" diet containing as high percentage as sucrose, the animals showed none of the symptoms, but attained normal growth, if they were administered daily with 10 γ vitamin B_1.

2. If 5 per cent of sucrose diet was replaced by the equal amount of molasses, the animals attained as good condition as those on "Kurozato" diet.

3. On the diet composed of 50 per cent sucrose plus 15 per cent "Ganmitsuto", the rats showed as healthy appearance as those on the latter two diets.

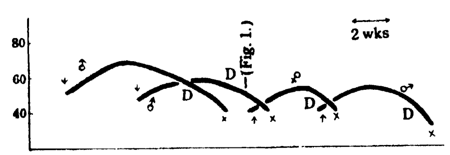


Chart 1. Growth curves of rats fed on sucrose diet (65%).
↓ or ↑ ······ B_1. D······dermatitis. × ······death.

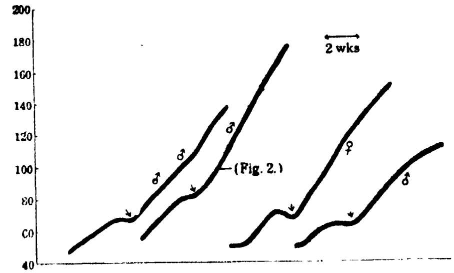


Chart 2. Growth curves of rats fed on "Kurozato" diet (65%).
↓ ······ B_1.

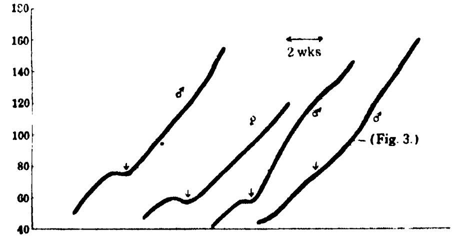


Chart 3. Growth curves of rats on molasses diet (60% sucrose + 5% molasses).
↓ ······ B_1.

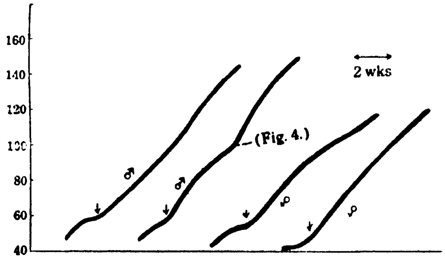


Chart 4. Growth curves of rats on "Ganmitsuto" diet (50% sucrose + 15% "Ganmitsuto").
↓ ······ B_1.

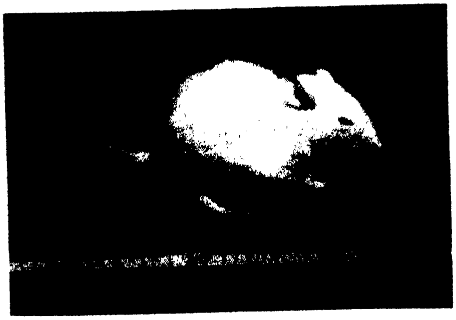


Fig. 1. Showing the dermatitis resulting from sucrose (65%) as the basal carbohydrate.

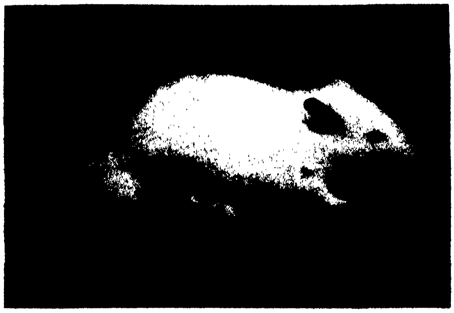


Fig. 2. Showing the healthy condition of the rat fed on "Kurozato" diet (65%).

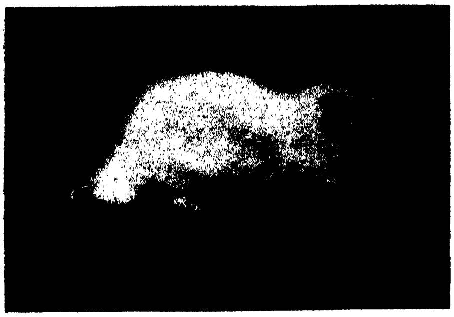


Fig. 3. Showing the normal growth of the rat fed on "Ganmitsuto" diet (Sucrose 50+ "Ganmitsuto" 15%).

Fig. 4. Showing the normal growth of the rat on molasses diet (Sucrose 60+ Molasses 5%).

These rats were litter mates. They were photographed on the thirty-ninth day of experiment, at which they weighed 52 g (Fig. 1), 98 g (Fig. 2), 97 g (Fig. 3), and 100 g (Fig. 4), respectively.

Part II.

In the preliminary experiment (*Part I*) we found that, in the rations mentioned above, if "Kurozato", "Ganmitsuto", and molasses were totally or partially substituted for sucrose, the animals on these diets did not develop the pellagra-like symptoms as in the case of sucrose diet, but attained normal growth.

Recently György(4) reported that crude cane molasses is good source of vitamin B_6 and also contains small amounts of flavin,

In this experiment we tried to find if the preceding crude sugars and molasses contain flavin, and also to separate the active factors essential for the nutrition of rats.

METHODS.

The growth of rats and the protection of the characteristic dermatitis of the dietary deficieney were employed in the assay of the fractions. The animals were placed on experiment in groups of from 4 to 6 animals each, care being taken in their group distribution in order to minimize both litter and sex variations.

The basal rations used in this experiment were similar to those described in *Part I*, but free from flavin, and had the following composition: Purified fish protein 20, Sugars 65, Crisco 3, Agar-agar 2, and McCollum's salt mixture 5, provided with 1 drop cod liver oil daily.

The rats received the rations deprived of flavin, had usually ceased to grow after two weeks, even though they were administered daily with 10 γ of vitamin B_1.

Preparation of supplementary materials—Separation of the active fractions from the crude sugars and molasses was carried out according to the method described in the previous publication.(1) The materials were exhausted carefully with 88~90% alcohol several times to remove inert substances. The combined alcohol extracts were then concentrated in vacuo to a suitable volume. One part of this concentrate was stored for assay, the other was diluted with H_2O, acidified with

H_2SO_4 to PH 2.0, and then adsorbed on acid clay repeating three times. The adsorbates were combined and eluted by shaking with 0.2 N $Ba(OH)_2$, and Ba was removed with H_2SO_4. The final filtrate from acid earth adsorbate was treated with $Ba(OH)_2$ to remove H_2SO_4. Both the eluate and filtrate were stored for test.

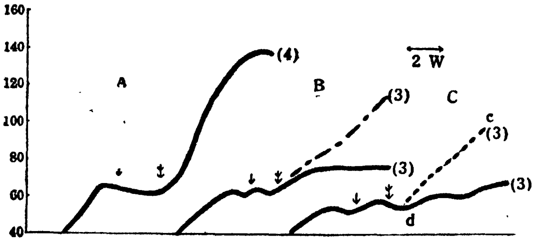


Chart 5. Growth curves of rats on sucrose plus molasses ration, and sucrose ration supplemented with the fractions from molasses. A······60% sucrose +5% molasses. B······65% sucrose and eluate of acid earth adsorbate (=5 g molasses) ; — — — — — added acid earth filtrate (=5 g molasses). C······65% sucrose and acid earth filtrate (=5 g molasses) ; — — — — added eluate of acid adsorbate (=5 g molasses).

↓······B_1. ‡······flavin. d······dermatitis. c······recovery.

The figures in brackets indicate the number of rats from which the growth curves were derived.

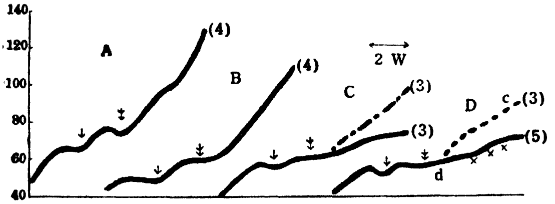


Chart 6. Growth curves of rats on "Kurozato" ration, and sucrose ration supplemented with the fractions from "Kurozato". A······65% "Kurozato". B······65% sucrose and concentrate of alcohol extract (=5 g "Kurozato"). C······65% sucrose and eluate of acid earth adsorbate (=5 g "Kurozato"); — — — — — added acid earth filtrate (=5 g "Kurozato"). D······65% sucrose and acid earth filtrate (=5 g "Kurozato"); — — — — added eluate of acid earth adsorbate (=5 g "Kurozato").

↓······B_1. ‡······flavin. d······dermatitis. c······recovery.

The figures in brackets indicate the number of rats from which the growth curves were derived.

RESULTS AND CONCLUSIONS.

The results with various sugars and their fractions with or without flavin were shown in Charts 5 to 7. Flavin was provided as dried egg white powder at the level of 6 per cent.

The results showed that "Kurozato", "Ganmitsuto", and cane molasses contained at least two factors essential for the rat growth. One factor, resembling vitamin B_6, was adsorbed on acid clay from the extracts of the sugars, the other was present in the filtrate after exhaustive treatment with acid clay. These sugars, however, did not contain vitamin B_1 and flavin as seen from the growth curves. We previously reported(1) that the vitamin B_2 requirement of rats was satisfied only when flavin, B_6 and an acid earth filtrate from liver extract were supplied together.

There is, at present, ample evidence that rats require for maximum growth four distinct factors of the vitamin B complex, namely, vitamin B_1, riboflavin, vitamin B_6, and the so-called filtrate factor.

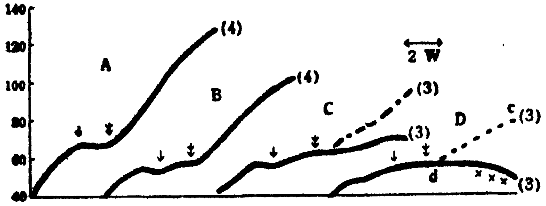


Chart 7. Growth curves of rats on sucrose plus "Ganmitsuto" ration, and sucrose ration supplemented with the fractions from "Ganmitsuto". A······50% sucrose +15% "Ganmitsuto". B······65% sucrose and concentrate of alcohol extract (=5 g "Ganmitsuto"). C······65% sucrose and eluate of acid earth adsorbate (=5 g "Ganmitsuto"); — — — — — added acid earth filtrate (=5 g "Ganmitsuto"). D······65% sucrose and acid earth filtrate (=5 g "Ganmitsuto"); — — — added eluate of acid earth adsorbate (=5 g "Ganmitsuto").

↓······B_1. ↡······flavin. d······dermatitis. c······recovery.

The figures in brackets indicate the number of rats from which the growth curves were derived.

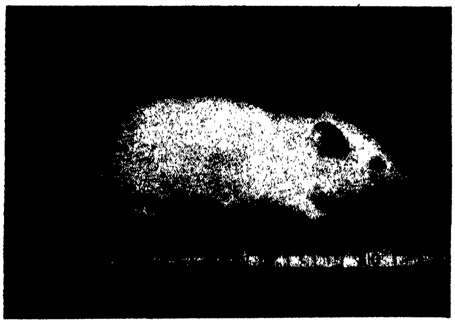


Fig. 5. Showing the influence of the concentrate of alcohol extract of "Kurozato" on the growth (body weight 97 g).

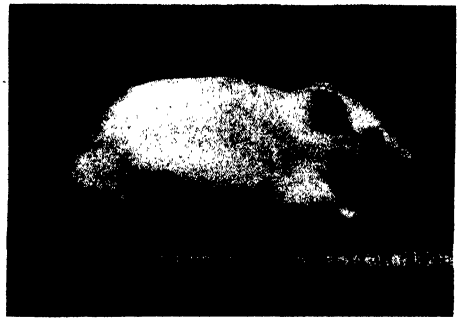


Fig. 6. Showing the influence of the concentrate of alcohol extract of "Ganmitsuto" on growth (body weight 102 g).

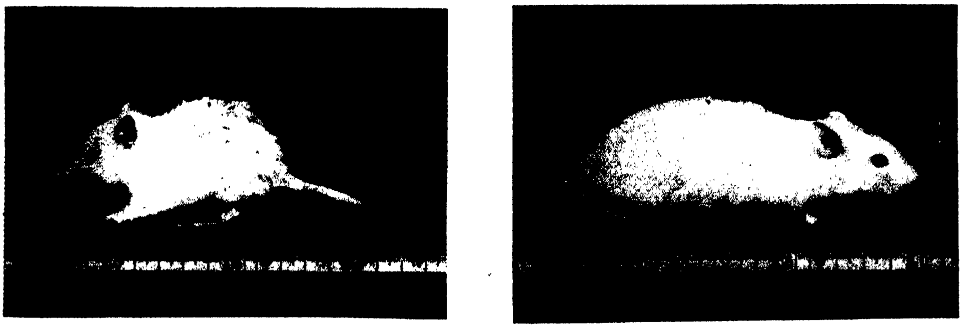


Fig. 7. (a)—Showing the inability of acid earth filtrate of cane molasses on preventing dermatitis caused by high sucrose diet (body weight 52 g).

(b)—Showing recovery of the rat (a) by supplementing eluate of acid earth adsorbate from molasses extract (body weight 88 g).

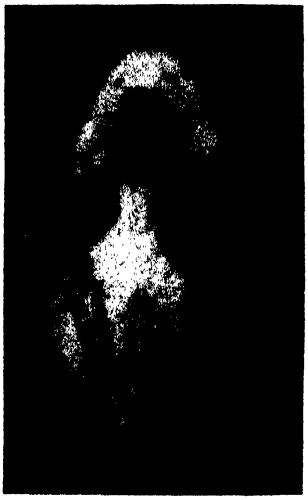
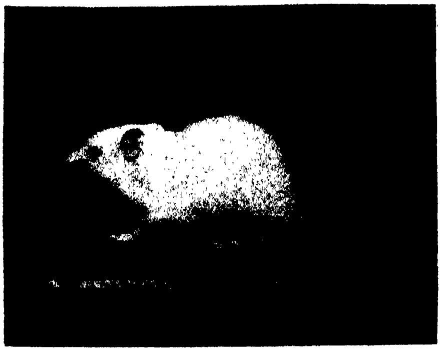


Fig. 8. (a)—Showing the inability of acid earth filtrate of "Ganmitsuto" to protect dermatitis due to high sucrose diet (body weight 50 g).



(b)—Showing recovery of the rats (a) by adding of eluate of acid earth adsorbate from "Ganmitsuto" extract (body weight 68 g).

SUMMARY.

1. When sucrose was used at the basal carbohydrate, the rats developed dermatitis, whereas if sucrose was totally or partially substituted by "Kurozato", "Ganmitsuto", or cane molasses, no dermatitis resulted.

2. The concentrate of alcohol extracts of the crude sugars mentioned above cured the dermatitis occurring in the rats fed on sucrose diet and promoted growth, provided adequate amounts of vitamin B_1 and flavin were fed simultaneously.

3. The data indicated that the concentrate of the crude sugars contained at least two factors essential for the nutrition of rats. One factor was adsorbed on acid clay, from which was eluted with 0.2 N $Ba(OH)_2$, the other was present in the acid earth filtrate; and the former may be identical with vitamin B_6 and the latter resembles the so-called filtrate factor.

We wish to thank Dr. U. Suzuki for his deep interest and helpful advice throughout this work. We are also indebted to Dr. T. Tanno for the analysis of samples.

(1) U. Tange: Rikagaku-kenkyu-jo Iho, **16**, 1058 (1937).
(2) T. W. Birch, P. György, and L. I. Harris: Biochem. J., **29**, 2830 (1935).
(3) T. H. Jukes and S. Lepkovsky: J. Biol. Chem., **114**, 117 (1936).
(4) P. György: Proc. Soc. Exp. Biol. and Med., **36**, 167 (1937).

Isolierung einer für die Vergärung des Zuckerrohrsaftes geeigneten Hefe aus dem Verdauungsorgan des Grashopfers.

Von Kano OHTSU und Kazuo YAMAFUJI.

(Aus dem Institut für Zuckerforschung in Tainan.)

Eingegangen am 9, Feb. 1939.

Gewöhnlich enthält der Verdauungssaft des Insekts verschiedene Mikroben. Die Enzyme dieser Mikroorganismen sind in einigen Fällen an den Verdauungsvorgängen beim Insekt beteiligt. In den anderen Fällen wird aber die Vitalität des Insekts durch das Toxin der Bakterien oder Pilze im Digestionssaft geschädigt. Wir haben im Zuckerrohrfeld einige Grashopfer gesammelt und das Gärvermögen der aus ihren Verdauungsorganen isolierten Hefen untersucht.

1. Isolierung der Hefen.

Nachdem man den Grashopfer mit 80% igem Alkohol desinfiziert hatte, wurde ihm das Verdauungsorgan entnommen. Von dem Inhalt dieses Organs brachten wir mittels einer kleinen sterilen Platinöse eine geringe Menge in den verflüssigten Kojiagar-Nährboden. Aus diesem Nährboden wurden in der gewöhnlichen Weise eine Reihe von Plattenkulturen hergestellt. Wir führten dann die Reinzucht der Hefe nach dem *Lindner*schen Tröpfchenverfahren durch. Weiter wurde die Hefe wiederholt auf dem Zuckerrohrsaft-Agar gezüchtet.

2. Ermittelung der Gärtätigkeit der isolierten Hefen.

Durch vorläufige Gärversuche wurden von den 18 Hefenarten, welche man in der oben angegebenen Weise aus dem Verdauungssaft des Grashopfers als Reinkulturen erhielt, zunächst 4 Sorten gewählt. Nachdem wurde dann die Gärfähigkeit dieser 4 Arten Hefen eingehender untersucht. Zu dieser Erforschung wurden die Hefen von der fünftägigen Zuckerrohrsaft-Agarkultur mit Hilfe eines Platindrahts in 50 ccm Saft aus dem 13 Monate alten Zuckerrohr gezüchtet. Den Gärverlauf stellten wir nach der früher mitgeteilten Methode (1) (2) fest.

Tabelle I. Eigenschaften des Saftes vor dem Vergären.

Grad Balling	12.26	Gesamtstickstoff in %	0.043
Reduzierender Zucker in %	1.10	Asche in %	0.23
Rohrzucker in %	8.61	pH	4.9
Gesamtzucker in %	10.15	Azidität	0.3

Azidität bedeutet ccm *n* NaOH pro 100 ccm Nährlösung.

Tabelle II. Gärverlauf.

Art der Hefe	Nr. 1	Nr. 2	Nr. 3	Nr. 4
Gesamtgärzeit in Stdn.	96	96	120	120
Entstandener Alkohol in Volum %	6.00	6.08	4.54	5.31
Entwickelte Kohlensäure in g	2.32	2.39	1.75	1.98
Endvergärungsgrad in %	91.75	92.96	69.41	81.19

Aus der Tabelle II geht hervor, dass die Hefe Nr. 2 die stärkste Gärwirkung hat. Wir nennen diese Hefe vorläufig „Saccharomyces batta".

3. Eigenschaften von „Saccharomyces batta".

a) Zellform und Zellgröße. Die im Zuckerrohrsaft von 12° Bg gezuchtete Hefe ist kreisförmig oder ellipsoidisch. Der Durchmesser dieser Hefe beträgt 3.5~5.5 μ.

b) Riesenkolonie. Bei der Zucht von Saccharomyces batta auf der Oberfläche des Zuckerrohrsaft-Agars entsteht eine Riesenkolonie, welche einen hervorragenden Kern und einen unebenen Rand besitzt.

c) Zuckerrohrsaft-Kultur. Während der Vergärung der Zuckerarten im Zuckerrohrsaft bleibt der Saft trübe. Nach der Gärung aber wird die Nährlösung klar.

d) Sporenbildung. Die vorher im Zuckerrohrsaft gewachsene Hefe bildet nach 20 Stunden bei 33° auf dem Gipsblock ihre Sporen aus.

e) Temperatur. Bei etwa 33° zeigt die Hefe die stärkste Gärtätigkeit sowie Vermehrungsfähigkeit. Die Tötungstemperatur der Hefe liegt bei 57°.

f) Wasserstoffionenkonzentration. Die Hefe vermehrt sich am besten bei pH 3.5~5.6.

4. Gärversuche in der Fabrik.

Versuch 1. 12 Tonnen des 18 Monate alten Zuckerrohrs (Rasse: F 108) wurden unter Anrühren mit Wasser ausgepresst. Der erhaltene Saft betrug 120 hl. Der Saft wurde in einem Gärbottich durch Erhitzen auf etwa 100° sterilisiert und dann wurde der Bottich mit Wasser auf 34° abgekühlt. Die Analyse dieses Saftes gibt folgende Resultate (Tabelle III).

Tabelle III.

Grad Balling	14.36	Gesamtstickstoff in %	0.047
Reduzierender Zucker in %	1.60	Asche in %	0.27
Rohrzucker in %	11.00	pH	5.2
Gesamtzucker in %	13.18	Azidität	0.8

Die Überführung der Hefereinkultur aus dem Laboratorium in den Betrieb erfolgte in der folgenden Weise:

Von einer fünftägigen Zuckerrohrsaft-Agarkultur wurde Saccharomyces batta in 50 ccm Zuckerrohrsaft von 15.62° Bg übergeimpft. Nach 48 stündigem Stehen bei 33° wurde die Hefe von neuem in 500 ccm Zuckerrohrsaft im *Pasteur*-Kolben weiter gezuchtet. Der frisch beimpfte Kolben wurde hierauf in den Thermostat von 33° gebracht und nach 27 Stunden wurde die erhaltene Hefesuspension in einen 150 hl fassenden Garbottich, der mit 120 hl Zuckerrohrsaft beschickt wurde, eingefuhrt. Fur 120 hl Saft wurden 5 *Pasteur*-Kolben verwendet. Dann wurde bisweilen die Luftung der Wurze ausgefuhrt.

Der Garverlauf ist in Tabelle IV wiedergegeben.

Tabelle IV.

Saft nach dem Vergaren		Gärung	
Grad Balling	−1 5	Gesamtgärzeit in Stdn.	52
Gesamtzucker in %	0 08	Entstandener Alkohol in Volum-%	8 08
pH	3 6	Endvergärungsgrad in %	95 17
Azidität	3 8		

Versuch 2. Aus 10 Tonnen des 13 Monate alten Zuckerrohrs (Rasse: 2725 POJ) erhielten wir durch Pressung 93 hl Saft. Die Eigenschaften dieses Saftes zeigt Tabelle V.

Tabelle V.

Grad Balling	12 73	Gesamtstickstoff in %	0 043
Reduzierender Zucker in %	2 74	Asche in %	0 30
Roh zucker in %	7 39	pH	5 1
Gesamtzucker in %	10 51	Azidität	1 9

Die Uberimpfung der Hefe in den Garbottich wurde im wesentlichen in der obenerwahnten Weise durchgefuhrt. Am Ende der Garung wurde der Saft analysiert (Tabelle VI).

Tabelle VI.

Saft nach dem Vergären		Gärung	
Grad Balling	−1 0	Gesamtgärzeit in Stdn	46
Gesamtzucker in %	0 138	Entstandener Alkohol in Volum-%	6 44
pH	3 7	Endvergärungsgrad in %	95 13
Azidität	4 3		

Zur Feststellung des Garvermögens von „Saccharomyces batta" mussen die Versuche im Fabrikbetrieb noch wiederholt angestellt werden.

Zusammenfassung.

Aus dem Verdauungssaft des Grashopfers im Zuckerrohrfeld wurde eine Art Hefe isoliest, die den Zuckerrohrsaft sehr stark zu vergaren vermag.

Literatur.

1) K. Yamafuji, K. Ohtsu u Y. Iwata: Biochem. Zs, **296**, 289 (1938).
2) Y Iwata, S Suzuki u K. Yamafuji: Bull. agricult. Chem. Soc Japan, **14**, **111** (1938).

Some Relations between Morphology and Physiology of *Aspergillus Oryzae*.

By Yoshio OTANI.

From the Zymomycological Institute, the Imperial College of Agriculture, Tottori, Japan.

(Received Feb. 17, 1939.)

INTRODUCTION.

Aspergillus Oryzae is a fungus which has an important part in brewing saké, shōchū (Japanese spirits), soy sauce and miso (soy bean paste). In 1878, Ahlburg(1) gave it the name *Eurotium Oryzae*. Later Cohn(2) revised the name to *Aspergillus Oryzae*, which is the current term used now. But at that time, the morphological description was very simple, and was not sufficient to show the characteristics of this species. Later, Büsgen(3) made a more careful study of the subject and showed the characteristics of this organism more clearly. Wehmer(4) isolated a strain of this fungus, through the pure culture method, and made a careful study of it. Thus the micro-biological characteristics were first shown in detail. Also, Klöcker and Shiönning(5) found that this fungus produced sclerotia. Studies of the enzymes of this fungus have been made by Atkinson(6); Kellner, Mori and Nagaoka(7); Stone and Wreight.(8) But a comparative study of the details of the morphological and physiological characteristics of the fungi, given in the studies made by these scholars, showed that there were a few points on which they disagreed. Takahashi(9) took some strains which he had isolated from moyashi (koji-flora) and made comparative experiments with these, and showed clearly that this species could be classified into a number of varieties. Oshima(10) found that from the enzymatic standpoint, the fungus could be divided into some groups and the groups classified according to the degree of enzymatic action in each. Recently, Sakaguchi(11) classified them according to their ability to produce metabolic products, such as alcohol and various acids. He(12) also made a classification, according to the colour and form of the mycelia of the fungus. Thus we see that *Aspergillus Oryzae* is only one species, but has in it groups which have some different characteristics.

The author has collected from various places, numerous strains of this fungus, and made a study of their morphology and physiology, trying to get a clearer knowledge of the relation between the two, the results of which are given below.

METHODS OF EXPERIMENT AND OBSERVATION.

Morphological studies :— Various strains of *Aspergillus Oryzae*, were cultivated on koji-agar at the temperature of 30°C, for two whole days, and the height of the mycelia mass, the diameter of the conidium, the length and diameter of the sterigma, the diameter of globule, the length and diameter of the conidiophore, and the width of the hyphae were examined both with the naked eye and under the microscope.

Physiological studies :— A study was made of the starch-decomposing power, protein decomposing power, the acid producing quality and the colour which was shown. The essential points and the methods are as follows :—

To measure the starch-decomposing power the medium was made through Peffer-solution, which has no cane sugar in it (the solution has 1% of ammonium nitrate, 0.5% of potassium bi-phosphate, 0.25% magnesium sulphate, a trace of iron chloride). Soluble starch was added as a carbon source in the following ratio, *i. e.*, 0.7%, 1.4%, 2.2%, 3.2%, 5% and 8%, and in these media the numerous strains of the fungi were inoculated. By the use of the iodine method, the degree of starch digested was noted day by day. If the strain had great power to decompose starch, when put into the incubator for 3 days, at the temperature of 30°C, the starch in the 0.7% soluble starch medium was digested. In the medium which had 8% of starch, there was no strain which was powerful enough to change it. In the medium which had 5% starch, there were 7 strains which changed it to a blue-violet colour, through the reaction of iodine.

Koji-gelatine was used to measure the power to decompose protein. 20% of gelatine was put into the koji-extract, and allowed to congeal. And in this, as a medium, strains of *Aspergillus Oryzae* was inoculated, and was left at the room temperature (about 20°C) for from three to five weeks. The strain which had a strong protein decomposing power quickly liquefied the gelatine. Thus it could easily be seen that the medium divided itself into three parts, mycelia mass, the liquefied part, and the congealed part. When the liquefied part were seen to be large, it was clearly seen that the protein decomposing power was great.

To measure the acid producing power, koji-extract was used. In this medium the strains were inoculated, and were cultivated for fifty days. Finally 5 cc of the metabolic solution was taken and was neutralized with $N/10$ NaOH solution, using phenolphthalein as an indicator. Through the amount titrated, the degree of acidity was determined.

The power to produce pigment was determined as follows :— the strains were inoculated into koji-extract or koji-agar, and cultivated for five weeks, and with the naked eye, the degrees of the developing dark brown colour were noted.

RESULTS OF THE EXPERIMENT AND DISCUSSION.

Through these experiments, it was found that there are a great many minor differences in the various strains, both in their morphology and in their physiology. If these differences are minutely classified, from the enzymatic standpoint, both the starch-decomposer and the protein decomposer might be divided into two groups. In addition, from the metabolic standpoint, it may be divided into acid-producer and pigment producer. The author took five strains each from the strongest strains, and gave the averages of these strains in the following table :—

	Enzymatic classification		Metabolic classification	
	Starch-decomposer	Protein-decomposer	Acid producer	Pigment-producer
Height of mycelia mass (mm)	3.06	1.90	3.1	1.94
Diameter of conidium (μ)	4.8~9.2	4.3~7.2	4.8~8.1	4.6~7.7
Sterigma length (μ)	13.9~27.6	13.8~19.2	15.1~23.4	12.2~19.3
Sterigma diameter	4.3~5.4	3.8~5.2	4.5~6.0	4.8~6.0
Diameter of sterigma (μ)	22.0~39.9	16.1~26.2	19.2~26.9	18.7~27.0
Conidiophore length (μ)	1.1~1.3	0.5~0.6	0.5~1.1	0.5~1.0
Conidiophore diameter (μ)	8.3~13.2	5.8~11.0	7.0~12.9	6.1~10.6
Width of hyphae (μ)	3.5~9.0	4.9~8.9	3.8~11.2	3.3~10.8
Decomposition of starch	++±	±	+	−
Decomposition of protein	±	+++±	±	+
Production of acid (cc)	3.28	2.84	5.73	2.17
Pigment formation	±	±	+±	+++

Note — − = 0, ± = few, + = the relative amount (indicated by the number of +)

As shown in the foregoing table, the greatest morphological difference shown was the height of the mycelia mass. The next greatest difference was in the diameter of the globules and in the length and diameter of the conidiophore. Comparing the morphological differences and the physiological differences, it was found that there was a direct relation between the starch decomposing power and the protein-decomposing power, between the acid-producing power and pigment-producing power.

For example, there are many points of similarity, between the starch decomposer and the acid producer, in the following points — the height of the mycelia, the diameter of the globules and the length and diameter of the conidiophore. Also the protein decomposer and the pigment producer were found to be very similar, in the morphology of the three parts mentioned above. From the physiological point of view, in the starch decomposer and in the acid producer, the starch-decomposing power and the acid producing power were found to be great. In the protein decomposer and in the pigment producer, the protein decomposing power was great. But in pigment production, the above mentioned relations did not hold good. So this side of the subject must be studied somewhat more thoroughly.

From the above facts, both the starch decomposer and the acid producer have an effect on carbohydrates. With regard to this, both the protein decomposer and the pigment producer seem to have an effect on the protein and its decomposition products. But the mechanism by which the dark brown colour appears in the *Aspergillus Oryzae* culture is not yet clearly understood, nor is the real construction of the pigment yet understood. So the author is not yet able to state any clear solution beyond that the above facts were noticed. In the various strains which the author used, there are some which seem to have both characters and there are some which seem to have a character between the two. But such variations seem to be a general characteristic of any micro-organism.

SUMMARY.

The author made a number of comparative experiments with regard to the morphology and physiology of various strains of *Aspergillus Oryzae*. The enzymatic characteristics may be divided into two, *i. e.*, starch decomposer and protein decomposer. There were two characteristics of the metabolic product, *i. e.*, acid producer and pigment producer. The morphology and physiology of the starch decomposer and the acid producer were very similar. Those of the protein decomposer and pigment producer were also very similar. Thus, we were able to place the starch decomposer and the acid producer in one group which has the power to act on carbohydrates. Both the protein decomposer and the pigment producer were classified in the other group which acts on protein and on its decomposition products. In addition, among the various strains, there were some which were between the two classes mentioned above, and there were some which had the characteristics of both groups.

Bibliography

(1) Ahlburg & S. Matsubara: Tokyo Ijishinshi, **24**, 12 (1878)
(2) F. Cohn: Jahresber. schles. Gesell. vaterl. Cultur, 61 (1883), 226 (1884)
(3) M. Büsgen: Ber. d. deutsch. Bot. Ges. 3, Generalvers. 66 (1885)
(4) C. Wehmer: Centralbl. Bakt., II, **1**, 150 u. 209 (1895)
(5) A. Klocker, u. H. Schiönning: Centralbl. Bakt., II, **1**, 782 (1895)
(6) R. W. Atkinson: Memoir, Tokyo Imp. Univ. Sc. Dept., **6**, 1 (1881)
(7) O. Kellner, Y. Mori, u. M Nagaoka: Ztschr. f. phys. Chem., **14**, 297 (1890)
(8) W. E. Stone & H. E. Wreight: J. Amer. Chem. Soc., **20**, 637 (1898)
(9) T. Takahashi: Jōzōshikenjo-Hōkoku, **12**, 121 (1906), **37**, (1911)
(10) K. Oshima: J. Coll. Agr. Hokkaido Imp. Univ., **19**, 135 (1928)
(11) K. Sakaguchi: Jōzōgaku-Zasshi, **8**, 241 (1930), **10**, 16 (1932)
(12) K. Sakaguchi: Jōzōronbunshū, III, 247 (1933)

ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

On the Retting of Vegetable Fibre Materials.

Part VIII. The Useful Anaerobes for the Bacterial Retting of Hemp Fibre Materials.

(pp. 207~212)

By Hideo KATAGIRI and Tosio NAKAHAMA.
(Agr. Chemical Laboratory, Kyoto Imperial University and Kanebo Yamasina Institute; Received Feb. 2 1939.)

Nearly the same effective retting of hemp fibre materials was attained by the anaerobic process as was previously observed by us with the aerobic bacteria (see Part VI & VII), and ten strains of anaerobic bacteria were isolated from the retting vat.

When pure fermentations of hemp fibre materials were carried out with these ten strains of bacteria, one strain of bacillus and one strain of coccus were selected as the most useful organisms.

One of the useful anaerobes was classified as a new species as *Kurthia cannabis liquefaciens*, since the characteristics of the bacteria were found not to be the same as those of Kurthia zopfii Trevisan, in the liquefaction of gelatin and in the production of indole or the acid formation from sugars.

The other useful gram-positive anaerobe was found to reveal similar cultural characteristics to Micrococcus cremoris-viscosi Hammer and Cordes. However, some physiological natures were quite different, since slimy fermentation of milk was never observed, gelatin was liquefied in infundibuliform and indole was produced by the bacteria.

It was therefore concluded that this bacteria was also a new species and it was named *Diplococcus cannabis*.

Effects of certain Minerals on the Growth of Root Nodule Bacteria. (Part II)

(pp. 213~224)

By K. KONISHI, T. TSUGE and A. KAWAMURA.
(Institute of Agr. Chem., Imp. University, Kyoto; Received Jan. 18, 1939.)

It was shown that most of the minor elements detectable in root nodules or roots exerted no oligodynamic effects on the growth of the organisms, but Mn and Cr did stimulate alfalfa bacteria on nitrate media, as well as Li lupine bacteria on yeast water media. A beneficial effect of ashes of water extracts from tops of several crops, when added to the culture, might be ascribed to their mineral constituents in special proportions. It suggested that increasing amounts of Na-sulfate and adding Ca-salt to the basic media were desirable in so far as the development of the organisms was concerned.

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The Metabolic Products of Penicillium funiculosum Thom. (Part I.)

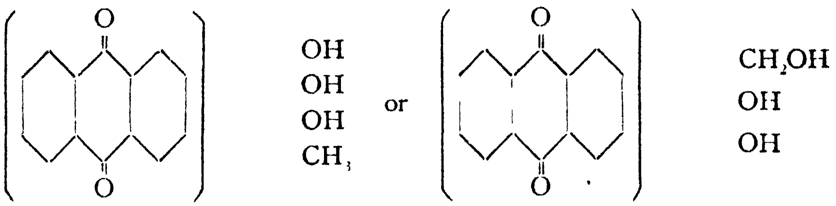
A Red Pigment, Funiculosin. (I).

(pp. 225~228)

By Hisanao IGARACI.

(Agricultural Chemical Laboratory, Tokyo Imperial University, Received Feb, 1,1938)

A mould fungus, Penicillium funiculosum Thom, was cultivated in Koji-decoction. After abont twenty days, the mycelium was gathered, treated with dilute NaOH solution, and filtered. To the clear violet solution so obtained, an excess of dilute hydrochloric acid was added. The red colored matter, which had precipitated, was dried, and extracted with light petroleum ether. On evaporating the petroleum ether from extract, red crystalline pigment, which was named funiculosin, $C_{15}H_{10}O_5$, m. p. 218° was obtained. Funiculosin yielded triacetyl (m. p. 205°) and tribenzoyl (m. p. 277°) derivatives, showing that funiculosin has 3 hydroxyl groups. By dry distillation of funiculosin with zinc dust, anthracen was obtained. By the reduction of funiculosin in alcoholic solution, the red color faded, but the color reappeared by the contact with air. Hence it was assumed that funiculosin was a quinonoid. From the results above described, the following formula was proposed for funiculosin.



The Metabolic Products of Penicillium funiculosum Thom. (Part II.)

Organic Acids.

(pp. 229~230)

By Hisanao IGARASI.

(Agricultural Chemical Laboratory, Tokyo Imperial University, Received Feb. 1, 1938)

Penicillium funiculosum Thom was cultured in Koji-decoction for 15~25 days. The mycelium was filtered off and the filtrate was evaporated to a small bulk, acidified with sulphuric acid and extracted with ether. After the evaporation of ether from extract, fine crystals of an organic acid which was identified with malonic acid, were isolated. When the synthetic medium, containing glucose and ammonium sulphate, was used in place of Koji-decoction, malonic acid together with succinic acid were obtained after 15 day's cultivation, but succinic acid only was obtained after 30 day's cultivation, and oxalic acid after 45 day's cultivation.

The Constituents of the crude Lecithin of Soyabean.

(pp. 231~234)

By Koji OKANO and Iwao BEPPU.

(The Central Laboratory of the South Manchurian Railway Co., Received Feb. 6, 1939)

Studies on Soyabean Oil-foots.

(III) Isolation of Flavin.

(IV) Manufacture of the Lactation-promoting Medicine and Tests of its Effects.

(pp. 235~242)

By Koji OKANO, Iwao BEPPU and Masatoshi OZU.

(The Central Laboratory of the South Manchurian Railway Co , Received Feb. 6, 1939)

Chemical Studies of Green Tea. 3.

On Pectin and on the Action of Pectinase on it.

(pp. 243~249)

By Saichi MACHIDA and Toshio TASHIRO.

(Laboratory of Shinsei-Shokuhin Co Ltd , Received Jan 24, 1939)

Decomposition Products of Substance containing Uronic Acid in Autoclave (IV).

Formation Mechanism of Alginetin.

(pp. 250~252)

By Kiyosi ASO.

(Tokyo Imperial University, Received Feb 13, 1939)

Studies on the Vegetable Tannins in Formosa. Part II.

Isolation of Tannin and its Diffusion Velocity into Gelatin-gel and Absorption Amount by Hide Powder.

(pp. 253~256)

By Yasuyosi OSIMA and Yôsuke KANEKO.

(Agricultural Chemical Department, Taihoku Imp. Univ, Taiwan, Japan,
Received Feb 13, 1939)

1) The raw materials reported in Part I were pulverized and repeatedly

extrated with warm water and the extract was saturated with salts and precipitate produced thereby was filtered off. The precipitate was dried in vacuo and extracted with aceton. From the extract, aceton was evaporated in vacuo and the residue was dissolved in warm ethyl acetate. Adding of chloroform into the ethyl acetate solution, tannin (A) was precipitated and there were dried in vacuo.

The filtrate of saturation with salt was shaken with ethyl acetate repeatedly and ethyl acetate extract was concentrated in vacuo and added an excess of chloroform. The precipitate of tannin (B) thus formed was dried in vacuo.

According to the original raw materials, tannins (A) and (B) was isolated respectively.

The results of analysis of isolated tannins were as follows:

Tannin	Soluble matter	Tannin	pH of 1% sol
Acacia decurrens (A)	87 4	83 7	3 9
Acacia confusa (B)	95 8	92 7	4 0
Areca catechu (B)	97 1	97 1	3 9
Thea sinensis (B)	95 3	94 2	3 3
Dioscorea cirrhosa (B)	93 0	90 1	3 9
Dioscorea Matsudai (B)	93 1	90 2	3 9
	95 6	93 4	3 2
	95 7	94 3	2 3
Therminalia chebula (A, B)	95 3	92 4	2 8
Gallotannin	91 1	84 4	3 3
d-catechin (m p 217)	101 0	90 1	4 2

The qualitative reaction of isolated tannins were entirely equal with the extract of raw materials and then the tannins were the main tannin in the raw materials.

2) Diffusion velocity into gelatin-gel. The hot solution contents 10% of gelatin and 0.1% of ferric chloride was coagulated in the glass tube of dia. 0.4 cm and length 10 cm. Take 15 cc of 1% of the tannin in the buffer solution of pH 4, 6, 8 respectively and into it the above glass tube was inserted for 72 hours at 25°C and the black colour tinted by the diffusion of tannin were measured.

The diffusion velocity was various according to the kind of tannin and in general pyrogallol tannin was fast and was faster in alkaline side.

Tannin	After 24 h			After 72 h.		
	pH 4	6	8	4	6	8
Gallotannin	1	5	10	2	7	17
Acacia decurrens	0	0	1	0	0	1
Acasia confusa	0	1	3	0	2	5
Areca catechu	0	0	4	0	0	4
Thea sinensis	0	4	6	1	5	10
Dioscorea cirrhosa	0	0	1	0	0	2
Terminalia chebula	7	12	16	11	15	23

3) Absorption amount of tannin by hide powder. Take 15 cc of 1% solution of the tannin in buffer solution of pH 2, 3, 4, 5, 6, 7, 8 and into it 0.1 g of hide powders were added and settled for 24 hours at 25°C. The hide powders were filtered off and washed until the reaction of tannin has disappeared and dried at 95°~100° and weighed. The amount of tannin absorped by the hide powder were as follows.

Tannin	pH 2	3	4	5	6	7	8
Acacia decurrens	33.0	22.6	13.6	17.9	23.3	23.5	23.9
Acacia confusa	14.2	15.6	18.9	16.7	12.6	16.7	13.4
Areca catechu	31.5	22.3	14.6	15.2	16.7	18.9	21.5
Thea sinensis	12.6	13.3	15.3	14.6	14.7	16.7	12.0
Dioscorea cirrhosa	27.0	22.1	17.5	18.6	19.5	21.2	22.7
Terminalia chebula	7.0	4.8	2.5	1.6	0.5	0.5	0.4
Gallotannin	35.6	25.8	17.6	14.3	17.5	21.6	27.0

The amount of tannin absorped by the hide powder was various according to the kind of tannin and in the case of Acacia decurrens, Areca catechu, Dioscorea and gallotannin the minimum point has existed at pH 4~5 and the maximum points were 2 and 8. In the case of Acacia confusa and tea, the minimum and maximum points has removed to neutral point.

Biochemical Studies on "Bakanae" Fungus of the Rice. Part II.

Isolation of "Gibberellin", the Active Principle which makes the Rice Seedlings grow slenderly.

(pp. 257~266)

By Teijiro YABUTA and Takeshi HAYASHI.

(Imperial Agricultural Station, Received Feb 6, 1939)

Kurosawa has reported that the seedlings grown in the sterilized filtrate of the culture solution of the "Bakanae" fungus show similar abnormal elongation to the so called "Bakanae", i.e. remarkably slender rice seedlings infected by the fungus naturally found in the rice nursery; hence he has suggested that the Bakanae phenomenon is caused by a toxic substance formed by the causative fungus.

The author attempted the isolation of the stimulus substance in crystalline state in order to make clear its chemical nature and mechanism of physiological action on the plant.

The successful procedure for the isolation of the active principle is as follows.

The most active strain among the Bakanae fungi is selected and cultured at 25°C for 30 days in the medium consisting of glycerine (3 g), ammonium chloride (3 g), acid potassium phosphate (3 g) and tap water (1000 cc), the pH of the solution being adjusted to 3.2 with hydrochloric acid. After that period the culture medium is filtered and sufficient amount of active carbon added to the filtrate with mechanical stirring; whereby the active principle is almost perfectly adsorbed. It is set aside overnight, the supernatant clear liquid then siphoned off, the sediment filtered by suction, washed with water and well drained on a Buchner's funnel. The adsorbate is now eluated with methanol-ammonia in the room temperature. To the eluate, after evaporation of the solvent under diminished pressure, basic lead acetate is added and the resulting precipitate is filtered off. The filtrate is treated with hydrogen sulphide and the filtrate from lead sulphide is reduced in vacuo to a small volume and extracted with ether in a soxhlet apparatus after addition of sodium bicarbonate; whereby basic, neutral and phenolic contaminations soluble in ether are removed. The extraction with ether is continued after acidification with sulphuric acid. On evaporating the ether extract and drying the residual syrup in vacuo over sulphuric acid, an almost colorless amorphous powder is obtained.

This substance thus prepared is effective for rice seedlings in quite minute amonuts, e. g. even in 0.00002% solution.

The authors propose the name "Gibberellin" for the active principle.

Gibberellin is easily soluble in alcohol, acetone and aqueous alkaline hydroxide or carbonate; fairly soluble in water and ether; difficultly so in chloroform, benzene and xylene; but insoluble in carbon disulphide, carbon tetrachloride and petroleum ether.

Gibberellin is quite labile in aqueous alkaline solution, losing its potency even in ordinary temperature. In acidic solution, however, it is rather stable.

Recently T. Yabuta and Y. Sumiki have succeeded in resolving the crude amorphous gibberellin into two different crystalline substances, both active for plants, by means of careful fractional crystallisation from ligroin mixed with ethyl-acetate or ethanol; one occurs as long prisms, m. p. 194∼6° [C% = 75.16 H% = 7.60 M. W. = 250 (by Rast)] for which the name Gibberellin A is provisionally proposed and the other occurs as short prisms, m. p. 245∼6° (decomp) [C% = 65.06, H% = 7.12, M. W. = 386 (by Rast), 357 (by titration as monocarboxylic acid) $[\alpha]_D = +36.13°$ (in methanol)] which has been termed Gibberellin B.

Studies on the Action of Arginase on Canavanin and Arginine III.

On the constitution of guanidine derivatives as the substrate for arginase.

(pp. 267∼279)

By Natsunosuke KITAGAWA.

(Biochemical Laboratory, Department of Agriculture, Kyushu Imperial University, Fukuoka; Received Feb, 3, 1939.)

On the Constituents of the Extract of Bamboo Sprouts.

(pp. 280～282)

By Teizo TAKAHASHI and Yoshinao YOKOYAMA.
(Agricultural College Gifu, Japan, Received Jan 23, 1939)

The following constituents were isolated from the water extract of bamboo sprouts (Phyllostachys mitis).

Oxalic acid	0 0533% of the material	0 1006% of the edible portion
K-tetraoxalate	0 0019	0 0037
Betaine	0 0097	0 0183
Tyrosine	0 0386	0 0729

On the Constitution of α-Sorigenin.

(pp. 283～290)

By Zirô NIKUNI.
(Agr Chem. Laboratory, Tokyo Imp Univ , Received Feb. 23, 1939)

α-Sorigenin was obtained as faintly yellowish fine needles from the bark of Kuroumemodoki (Rhamnus japonica, Max.). (Bull. Agr. Chem. Soc. of Japan: **14**, 25 (1938); **15**, 15 (1939)

It is soluble in alcohol, methanol and acetone but insoluble in water. Dilute alkaline solution dissolves it and the sunlight turns it to violet. It melts at 227～229° and its experimental formula is $C_{13}H_{10}O_5$.

The constitution of this compound was determined as follows:

A. Determination of five oxygen atoms.

1) Two free hydroxyl-groups.

The alcoholic solution of α-sorigenin turns to deep greenish brown by ferric chloride solution. It takes two acetyl-groups by acetylation with acetic anhydride and sodium-acetate, and takes also two methyl-groups by methylation with diazomethane. These derivatives have no colour reaction with ferric chloride solution.

2) One methoxyl-group by Zeisel's method.

3) Lactone ring.

By dilute caustic alkaline solution α-sorigenin and α-sorinin take one molecule of water and then precipitate as their monoalkaline salts. And these salts return easily to their original compounds by acid. Therefore the existence of γ or δ lactone ring was determined.

The new hydroxyl-group, produced by the breakdown of lactone ring, has no colour reaction with ferric chloride solution, and takes no methyl-group by the methylation with diazomethane. So it must be an alcoholic hydroxyl-group.

4) Carbonyl, methylendioxide, free carboxyl and carboxylmethylester-groups do not exist.

5) The existence of the following four compounds was tested by their characteristic colour reactions but they were all negative.

(a) 4′-Hydroxyflavone.
(b) Hydroxy-methyl-anthraquinone.
(c) Diphenylen-dioxide.
(d) Coumarin.

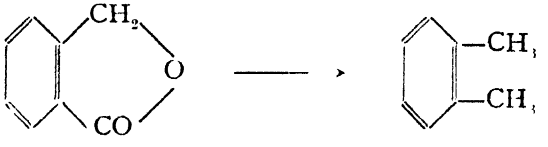
So the five oxygen atoms were all determined two as free hydroxyl, one as methoxyl, two as lactone ring.

B. Determination of naphthalene nucleus.

When heated with alkali *α*-sorigenin and their derivatives give out a naphthalene-like smell.

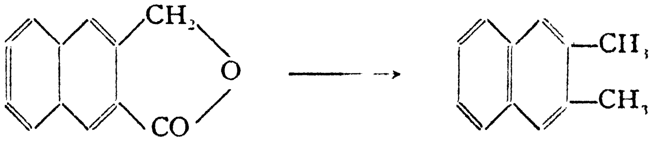
By the zinc dust distillation in hydrogen gas stream, diacetyl-*α* sorigenin gave a small quantity of yellowish oil with characteristic naphthalene-like smell. This oil separates from itself colourless plates on cooling below −10°. On recrystallization from dilute alcohol the colourless crystals melt at 90~96°. Its absorption spectrum resembles that of naphthalene, demonstrating that the crystals may be a derivative of naphthalene.

The analytical result of this crystal coincides very well with that of dimethyl-naphthalene $C_{12}H_{12}$. Among ten isomers of dimethyl-naphthalene, 2, 3; 2, 8; 1, 5; 2, 6 and 2, 7-dimethyl-naphthalenes were obtained as crystals, but in these isomers the probable form is only 2, 3 dimethyl-naphthalene (m p. 104°) from the positions of side chains.



Phthalide

o-Xylol



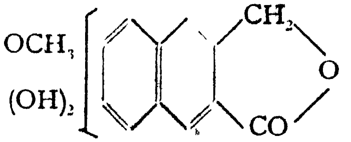
Lactone of 3-hydroxymethyl-*β*-naphthoic acid

2, 3-Dimethyl-naphthalene

From the fact that the phthalide gave *o*-xylol by zinc dust distillation, it is very probable that the lactone of 3-hydroxymethyl-*β*-naphthoic acid may give 2, 3-dimethyl-naphthalene by the same treatment.

From these experimental results and discussions the author concludes that *α* sorigenin may be the lactone of monomethoxy-dihydroxy-3-hydroxymethyl-*β*-naphthoic acid.

Methoxyl and hydroxyl groups may be combined with naphthalene nucleus, but their positions are not yet decided.



α-Sorigenin
=Lactone of monomethoxy-dihydroxy-3-hydroxymethyl-*β*-naphthoic acid

The author expresses his sincere thanks to Prof. B. Suzuki for his kind guidance throughout this work.

The Influence of Monochromatic Lights on the Action of Enzymes.

[Report XII.] Especially on the Influence of Ultra-violet Rays on the Action of Enzymes.

(Part 1.) Yeast Saccharase.

(pp. 291～299)

By Reitaro MURAKAMI.

(Agricultural College, Utunomiya, Received Jan 31, 1939)

In order to investigate the influence of ultra violet rays on the action of the saccharase in the yeast, the author has employed "Vim Ray" blue lamp. Enzyme solution was prepared as follows: After the liquefaction was completed by the addition of 10% toluene into 225 g of "Oriental" pressed yeast the same weight water was added and the yeast was allowed to autolyze at room temperature for 7 days. The precipitation obtained by adding an equal volume of alcohol to the autolyzed liquid was extracted by 20% alcohol. To the extract was then added alcohol and the second precipitate was extracted with 100 cc of water.

The author has added 1 cc of the enzyme solution into the test tubes containing 4 cc of 20% saccharose dissolved in 1% primary sodium phosphate solution and the pH of the solution adjusted to 4.24.

The test tubes were placed within the tin boxes, at the front windows of which were placed ultra-violet-close-filter and ultra-violet-pass-filter. The filters of white and black passing respectively ultra-violet and visible or no rays were also applied as controls. The boxes containing the test tubes were incubated at 40°C and the door opened, and lighted by the "Vim Ray" blue lamp through the layer of copper sulphate solution and filters. The transmission wave langths of each filter were as follows: white……3500～5500 I. Å., ultra-violet-close…… 4100～5500 I. Å., ultra violet-pass……3565～3984 I. Å., black……none.

The test tubes were taken out from each box at the intervals of 30, 60, 90 and 120 minutes after the incubation, and the each tube 2 cc of saturated Na-carbonate solution were added and the contents diluted to 250 cc with water and then the determination of invert sugar contents were made by Bertrand's method.

In this experiment, the action of the yeast saccharase was found to be inhibited by the ultra-violet rays and promoted by the visible rays The action of the enzyme was promoted at first under the influence of the rays containing both ultra-violet and visible, but the action of the enzyme was finally inhibited.

[Report XIII.] Especially on the Influence of Ultra-violet Rays on the Action of Enzymes.

(Part 2.) Yeast Amylase.

In order to investigate the influence of ultra-violet rays on the action of the amylase in the yeast, the author has employed "Vim Ray" blue lamp. Enzyme solution was prepared by the autolysis for one day at room temperature and then for overnight in an ice box after adding 20 cc of toluene and 500 cc of water into

200 g of "Oriental" yeast.

The author has added 10 cc of the enzyme solution and 10 cc of phosphate buffer solution to the test tubes containing 10 cc of 1% soluble starch solution, and the pH of the solution adjusted to 5.1. The tubes were placed within tin boxes, at the front windows of which ultra-violet tight filter, ultra-violet pass filter, white filter and black filter were placed. The boxes containing the test tubes were incubated at 40°C and lighted by "Vim Ray" the lamp through the layer of copper sulphate solution and filters. As to the qualities of the filters see the suthor's previous paper. [Report XII.]

The tubes were taken out from each box at the intervals of 8, 16 and 24 hours after the incubation and then the end product of the hydrolysis by the enzyme was determined by Bertrand's method.

In thir experiment, the action of the yeast amylase was found to be inhibited under the influence of ultra-violet rays and promoted by the visible rays. The action of the enzyme was also promoted by the rays containing both ultra-violet and visible rays.

Studien über Sericin. V. Mitteilung.

(ss. 300～304)

Von Takeo Ito und Kozo Komori.
(Aus dem chem. Laboratorium der Seidenbau-Hochschule, Kyoto,
eingegangen am 15. Feb. 1939)

In der vorliegenden Untersuchung wurden die bei dem Erhitzen im Wasser auftretenden Veranderungen der Sericinfraktionen weiter verfolgt. Sericin A wurde durch kurzdauernde Erhitzung bei 105° im Wasser gelöst. Je 50 ccm von dieser Lösung wurden unter den in Tab. 1 angegebenen Bedingungen erhitzt, nach dem Abkuhlen mittels eines Azetat-Puffers gepuffert und sodann auf 75 ccm aufgefullt. Hierauf hat man pH, Viskosität und das durch 50%-igen Alkohol koagulierbare Sericin-N bestimmt. Die Resultate sind in Tab. 1 zusammengestellt.

Tabelle 1. Die durch Erhitzung verursachte Verminderung der Viskositat und der Alkoholkoagulierbarkeit von Sercin-A-Losung.

Erhitzungs-		Konzentration (mg N/100 ccm)	pH	Relative Viskosität (25°)	Durch 50%-igen Alkohol koagulierbarer Sericin N (% gegen Gesamt-N)
Temperatur	Dauer				
——	——	100.3	3.87	1.093	85.70
100°	1 Stde.	99.5	3.86	1.086	80.19
110°	0.5 "	99.8	3.89	1.067	58.82
"	1 "	100.4	3.89	1.061	45.94
"	2 Stdn	100.6	3.93	1.058	26.51
120°	1 Stde.	100.3	3.88	1.061	28.47

Es ist zu ersehen, daß durch Erhitzung die Viskositat als auch die Alkohol-Koagulierbarkeit der Sericin-A-Lösung vermindert wird, und zwar die letztere in starkem Maße.

Auf Grund dieser Tatsache, zumal wenn man die damit gleichzeitig auftretende Ammoniak-Abspaltung, welche in einer früheren Arbeit festgestellt worden ist, in Betracht zieht, so ist es leicht annehmbar, daß sich die Micelle von Sericin A bei höheren Temperaturen irreversibel desaggregiere und dadurch verkleinere.

Versuche mit Sericin B wurden folgendermaßen durchgeführt: Verschiedene Mengen von Sericin B wurden je mit 50 ccm Wasser eine Stunde lang bei einer gegebenen Temperatur erhitzt. Dabei ging Sericin B stets in teilweise Lösung über. Die Lösung wurde noch gelinde warm samt dem ungelösten Teil in einen Meßkolben eingegossen, nach dem Abkühlen mit 10 ccm von 0.2 molarer Azetatpufferlösung versetzt, dann auf 100 ccm aufgefüllt und unter Toluol-Zusatz bei 25° über Nacht stehen gelassen. Mit dem aliquoten Teil wurde der aufgelöste Stickstoff nach Kjeldahl bestimmt.

Beistehende Abb. 1 gibt die erhaltenen Resultate graphisch wieder, wobei auf der Abszisse die beim Versuche angewandten Mengen von Sericin B in Gramm, und auf der Ordinate die Mengen vom aufgelösten Stickstoff in Milligramm aufgetragen wurden. Sie zeigt, daß Sericin B annähernd proportional mit dessen Menge, welche beim Versuche angewandt wird, in Lösung geht. Analoge Tatsachen sind bereits vorlängst von Hardy und Mellanby (1905) gefunden worden. Nach ihren Versuchen ist nämlich die bei einer bestimmten Salzkonzentration gelöste Globulinmenge mit derjenigen Globulinaufschlämmungsmenge, welche bei dem Lösungsversuche in Arbeit genommen wurde, annähernd proportional. Durch umfassende Versuche konnte Sörensen Koll. Z. 53, 110 (1930) nicht nur die Beobachtung der genannten Forscher bestätigen, sondern er konnte noch beweisen, daß eine solche partielle Lösung oder eine partielle Fällung vom Globulin eine Fraktionierung des Globulins herbeiführt. Nach ihm kann diese Tatsache dadurch einfach erklärt werden, daß man sich die Globulin als leicht dissozierbare Verbindungen, nicht aber als Mischungen zweier oder mehrerer Fraktionen vorstellt. Er nimmt weiter an, daß sowohl der undissozierte Globulinkomplex, als auch die daraus gebildeten Dissoziationsprodukte höchst verschieden in Salzlösung löslich seien. Wird ein solcher Globulinkomplex mit einer Salzlösung, die zur vollständigen Lösung des Globulins nicht hinlänglich konzentriert ist, behandelt, so werden nur die am leichtesten löslichen Dissoziationsprodukte in Lösung gehen. Bei Anwendung von größeren Globulinmengen, werden sich dementsprechend größere Mengen von den löslichen Dissoziationsprodukten auflösen.

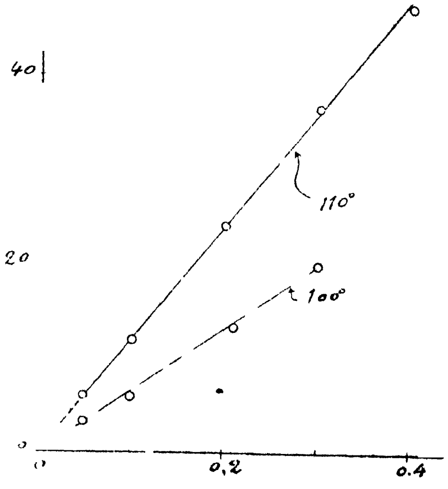


Abb. 1.

Auf diese Weise erhält man eine einfache Erklärung für das angeführte sonderbare Auflösungsverhalten des Globulins.

Die Auflösung von Sericin B in heißem Wasser muss indessen etwas anders aufgefasst werden, denn hier handelt es sich zweifellos um einen irreversibelen Vorgang, welcher von dem reversibelen Vorgang wie im Falle des Globulins scharf getrennt gehalten werden muß, was auch schon von Sörensen (loc. cit.) selbst nachdrücklich betont worden ist.

Wahrscheinlich wird sich Sericin B bei der Behandlung mit Wasser bei höheren Temperaturen irreversibel desaggregieren (bzw. spalten), und eine oder mehrere von den Desaggregationsprodukten werden in Lösung gehen. Für diese Deutung spricht auch, daß der dabei ungelöst zurückbleibende Anteil anders zusammengesetzt ist als das zuerst zum Versuche angewandte Sericin B, wie aus der folgenden Tabelle zu ersehen ist.

Tabelle 2.

	Gehalt an Kohlenhydrat in mg pro 100 mg Sericin-N		Verteilung vom Stickstoff				
	Zucker (als Glukose berechnet)	Aminozucker (als Glukosamin berechnet)	Ammoniak-N	Melanin-N	Diaminosäure-N	Monoaminosäure-N	Summe
Sericin A	1.8	1.9	12.14	0.17	16.56	70.10	99.58
Sericin A_1	2.4	3.5	10.89	0.85	17.47	70.53	99.88
Sericin B	7.3	7.5	10.22	0.90	18.20	69.19	99.65
Sericin B_1	15.9	13.2	9.34	1.16	20.01	68.96	99.47

In Tab. 2 bedeutet Sericin A_1 den in heißem Wasser aufgelösten und nachher durch Alkohol-Zusatz (50%) ausgefallenen Anteil von Sericin B, und Sericin B_1 denjenigen Anteil, welcher ungelöst zurückblieb.

Es wird oft ausgesprochen, daß sich das Sericin B bei höheren Temperaturen allmählich in das Sericin A umwandele. Unserer Erfahrung gemäß, dürfte indessen diese Aussage nicht dahin ausgehen, daß, wie oben erwähnt durch irreversibele Desaggregation von Sericin B ein wasserlöslicher Anteil entsteht.

Ferner spricht man auch von der Wichtigkeit der Kenntnisse bezüglich der Verteilung der Sericinfraktionen in der Kokonschicht für die „Filature" Chemie. Um diese Verteilung experimentell zu ermitteln, verfahrt man gewöhnlich in der Weise, daß man den Sericin-Auszug, welcher aus Kokonschicht durch Behandlung mit heißem Wasser gewonnen wird, nach einem oder anderem Verfahren analysiert. Es ist aber aus dem oben Gesagten leicht ersichtlich, daß bei den Bedingungen der Ausziehung des Sericins namentlich die Temperatur auf die Analysierungsresultate starken Einfluß ausüben. Als Beispiel hierfür seien die Resultate eines von unseren Versuchen angeführt. Aus der Kokonschicht von derselben Art wurde das Sericin sonst bei gleichen Bedingungen einmal bei 110°, und das andere Mal bei 120° mit Wasser ausgezogen. Der gesamte N in den beiden Auszügen betrug 3.677 g resp. 3.798 g pro 100 g Kokonschicht. Sie wurden nach der von uns modifizierten Mosherschen Methode analysiert. Auf diese Weise ergab sich der Zahlenwert für Sericin-B-N im ersten Falle zu 60.09% und im zweiten Falle zu 27.42% vom gesamten N, also besteht ein ausserordentlich großer Unterschied zwischen den beiden Resultaten.

. 15 April 1939 No. 4

Bulletin of the Agricultural Chemical Society of Japan.

TRANSACTIONS

Über Zuckerbestimmungen im Zuckerrohrsaft.

Von T. Yoshida, T. Tanabe und K. Yamafuji.

Aus dem Institut für Zuckerforschung in Tainan.

(Eingegangen am 10 März 1939.)

In der vorliegenden Arbeit haben wir einige Versuche über die Bestimmungen bzw. Veränderungen des Zuckergehaltes des Zuckerrohrsaftes angegeben.

I. Zur Bestimmungsmethode der Zucker im Zuckerrohrsaft.

Bei der Bestimmung des reduzierenden Zuckers im Zuckerrohrsaft ist der Einfluß der darin befindlichen Saccharose auf die Genauigkeit der Zuckerbestimmung zu berücksichtigen. Bekanntlich wird der Rohrzucker durch Behandlung mit Reagenzien, welche bei der Monosebestimmung gewöhnlich verwendet werden, einigermaßen invertiert. In einer früheren Veröffentlichung (1) haben wir zwei Methoden berichtet, die beim Vorhandensein einer großen Menge Rohrzucker die reduzierenden Zucker zuverlässig bestimmen lassen. Diese Methoden wurden später ergänzt und verbessert (2). Wir haben auch eine Mikromethode ausgearbeitet, welche die Bestimmung einer sehr geringen Menge Zucker ermöglicht (3). In der vorliegenden Untersuchung wurde zunächst versucht, die Ofnersche Methode (4), die oft zur Bestimmung des reduzierenden Zuckers in rohem Rohrzucker benutzt wird, zur Ermittelung des Zuckergehaltes des Zuckerrohrsaftes anzuwenden.

1 ccm frisch gewonnenen Zuckerrohrsaftes wurde mit 0,2 ccm 10%iger Bleiacetatlösung versetzt, geschüttelt und mit Wasser auf 25 ccm verdünnt. Diese Lösung wurde filtriert und zu 15 ccm Filtrat 0,5 ccm einer 10%igen Natriumphosphatlösung zugesetzt. Wieder filtrierten wir die Lösung und verwendeten das erhaltene klare Filtrat zur Zuckerbestimmung.

Zur Ermittelung der gesamten Zuckermenge muß die Saccharose im Filtrat mit Säure vollständig invertiert werden. Um die geeignetste Bedingung für die Inversion zu finden, wurden 50 ccm einer 0,2%igen Rohrzuckerlösung mit 10 ccm Salzsäure von verschiedenen Konzentrationen 30 Minuten im siedenden Wasserbad erhitzt, dann mit Natriumhydroxyd neutralisiert und der entstandene Invertzucker nach der Ofnerschen Methode bestimmt (Tabelle I). Da die Ofnersche Methode in einer schwer zuganglichen Zeitschrift veroffentlicht worden ist, wird hier das Verfahren kurz angegeben.

10 ccm der mit Natriumhydroxyd neutralisierten Losung werden mit 50 ccm Ofnerscher Kupfersulfatlosung versetzt. Man erhitzt die Mischung in 3 Minuten Kochen und kuhlt rasch in fliessendem Wasser ab. Dann setzt man 15 ccm 1 *N* Salzsaure langsam hinzu und anschließend noch 10 ccm 0,032 *N* Jodlosung. Nach 2 Minuten langem Stehen bei Zimmertemperatur wird die Mischung mit 0,032 *N* Thiosulfatlosung gegen Starke titriert. 1 ccm der verbrauchten 0,032 *N* Jodlosung entspricht immer 1 mg Invertzucker.

Fur die Zuckerbestimmung nach der Ofnerschen Methode sind folgende Reagenzien erforderlich:

1. 5 g kristallisiertes Kupfersulfat + 50 g kristallisiertes Natriumphosphat + 10 g wasserfreies Natriumcarbonat + 300 g Seignettesalz in 1000 ccm Wasser.
2. 0,032 *N* Jodlosung: 10 g Jodkalium + 2,05 g Jod in 500 ccm Wasser.
3. 0,032 *N* Thiosulfatlosung: 4 g Thiosulfat in 500 ccm Wasser.

Tabelle I.

Konz. der Salzsäure in *N*	Invertzucker in mg	Konz der Salzsäure in *N*	Invertzucker in mg	Konz der Salzsäure in *N*	Invertzucker in mg
0,01	2,74	0,2	4,15	4,0	3,85
0,02	3,58	0,5	4,20	6,0	3,45
0,05	4,08	1,0	4,15	12,0	2 65
0,1	4,10	2,0	4,00		

Bei 30 Minuten langer Erhitzung der Rohrzuckerlosung mit 0,2～1,0 *N* Salzsaure wird der Zucker fast vollstandig invertiert; die berechnete Menge Invertzucker betragt hierbei 4,21 mg.

Wir erhitzten dieselbe Saccharoselosung verschiedene Zeiten mit 1 *N* Salzsaure auf 100° und verglichen die gefundenen Invertzuckermengen (Tabelle II).

Tabelle II.

Erhitzungsdauer in Min	Invertzucker in mg	Erhitzungsdauer in Min	Invertzucker in mg
1	1,25	10	4,20
3	3,55	20	4,20
5	4,10	30	4,15

Aus den Versuchen in Tabelle II geht hervor, daß die vollkommene Inversion der Saccharose nach 10 Minuten langem Erhitzen auf 100° stattfindet.

Der reduzierende Zucker im Zuckerrohrsaft kann direkt nach der Ofnerschen Methode bestimmt werden. Zur Ermittelung des Saccharosegehaltes des Saftes muß man die Menge des reduzierenden Zuckers von der auf obenerwahnte Weise bestimmten Gesamtzuckermenge subtrahieren.

Ofner hat in seiner Mitteilung berichtet, daß nach seiner Methode 1 ccm der verbrauchten Jodlosung 1 mg Invertzucker entspricht; dabei wird die verbrauchte

Jodmenge dadurch berechnet, daß man den Titrationswert der 0,032 *N* Thiosulfatlosung von der vorher zugesetzten Menge des 0,032 *N* Jods abzieht. Es ist noch unbekannt, ob dieses Verhaltnis auch fur die anderen reduzierenden Zucker angewandt werden kann. Bekanntlich fallen die relativen Reduktionswerte der Zucker fur die verschiedenen Methoden sehr verschieden aus. Wir haben nun die Glucose in der obenerwahnten Ausfuhrungsweise bestimmt (Tabelle III).

Tabelle III.

Zugesetzte Glucose in mg	0,032 *N* Jod in ccm	Zugesetzte Glucose in mg	0,032 *N* Jod in ccm
2	1,92	6	6,00
4	3,94	8	8 11

Die Tabelle III zeigt, daß das von Ofner fur Invertzucker festgestellte Verhaltnis: 1 ccm 0,032 *N* Jod =1 mg Zucker sich auch auf die Glucose anwenden lasst.

Die Zusatzversuche in Tabelle IV dienen als Belege fur die Anwendbarkeit der Methode auf die Zuckerbestimmung im Zuckerrohrsaft.

Tabelle IV.

	Grade Balling	Rohrzucker in %		Reduzierender Zucker in %	
		Polarisationsmethode	Die von uns benutzte Methode	Luffsche Methode	Die von uns benutzte Methode
Saft Nr. 1	17,1	14,94	14,79	1,070	1,039
Saft Nr. 2	16,6	13,98	13,77	1,570	1,581
Saft Nr 3	16,9	14,46	14,23	1,660	1,665

II. Über die Veranderungen des Zuckergehaltes des Zuckerrohrsaftes beim Stehen.

Wenn der Zuckerrohrsaft bei Zimmertemperatur stehengelassen wird, so findet allmahlich eine Zersetzung der Zucker im Saft durch Enzyme oder Mikroben statt. Fur die Reihenanalyse des Saftes ist es notwendig, diese Zersetzung beim Stehen vollkommen zu verhindern. Da im allgemeinen in der Fabrik eine große Anzahl von Saften gleichzeitig analysiert werden mussen, haben wir in dieser Versuchsreihe uber die faulniswidrigen Mittel fur den Zuckerrohrsaft eingehend untersucht.

Die frisch geernteten Zuckerrohre wurden 24 Stunden bei Zimmertemperatur stehengelassen und dann mittels einer Presse ausgepresst. Der erhaltene Saft wurde in einen Erlenmeyer-Kolben gebracht, mit einer bestimmten Menge eines antiseptischen Mittels gemischt und alsdann der Kolben gestopselt. Nach bestimmten Zeiten wurde der Zuckergehalt des Saftes ermittelt. Dabei wurde der Rohrzucker nach der Polarisationsmethode und der reduzierende Zucker nach der Schoorlschen Methode (5) bestimmt.

1. *Mercurichlorid.*

Tabelle V.

Saft aus dem unreifen Zuckerrohr. Aufbewahrungstemperatur: 28°~31°. Brix des Saftes: 18.6°.

Aufbewahrungsdauer in Stdn.	0	6	24	0	6	24
$HgCl_2$ in g pro 1 *l* Saft	Rohrzucker in %			Reduzierender Zucker in %		
0	16,36	19,30	15,66	1,05	1,16	—
0,1	16,36	16,36	16,35	1,05	1,08	1,12

Tabelle. VI.

Saft aus dem reifen Rohr. Aufbewahrungstemperatur: 27°~29°. Brix des Saftes: 22,5°.

Aufbewahrungsdauer in Stdn	0	6	24			24
$HgCl_2$ in g pro 1 *l* Saft	Rohrzucker in %			Reduzierender Zucker in %		
0	21,09	21,04	20 85	0,29	0,41	0,90
0,250	21,09	21,09	21,07	0 29	0,32	0,32
0,100	21,09	21,09	21,07	0,29	0,32	0,33
0,050	21,09	21,09	21,07	0,29	0,32	0,33
0,025	21,09	21,08	21,00	0,29	0 32	0,33

Tabelle VII.

Saft aus dem überreifen Rohr. Aufbewahrungstemperatur: 28°~31°. Brix des Saftes: 14,2°.

Aufbewahrungsdauer in Stdn	0	6	24	0	6	24
$HgCl_2$ in g pro 1 *l* Saft	Rohrzucker in %			Reduzierender Zucker in %		
0	10,40	10,20	9,20	1,88	1,97	0,18
0,1	10,40	10,40	10,20	1,88	1,89	1,97

Wie aus den Tabellen V, VI und VII ersichtlich ist, wird die Zersetzung der Zuckerarten im Saft durch Zusetzen einer sehr geringen Menge Mercurichlorid vollständig verhindert. Wird das Mercurichlorid nicht zugesetzt, so wird der Rohrzucker allmählich invertiert und es vermehrt sich der reduzierende Zucker. Nach längerer Aufbewahrungszeit vermindern sich aber auch die Monosen. Diese Veränderungen der Zucker sind im allgemeinen beim Saft aus dem überreifen Zuckerrohr stärker ausgeprägt, als beim Saft aus dem unreifen Rohr.

2. *Formalin.*

Tabelle VIII.

Aufbewahrungsdauer in Stdn	0	6	24	0	6	24
	Rohrzucker in %					
Formalin in g pro 1 l Saft	Reifes Rohr			Uberreifes Rohr		
0	18,06	18,04	17,69	18 43	18,31	17,25
1,00	18,06	18,06	17,95	18,43	18,31	18,11
0,50	18,06	18,06	17 95	18,43	18,35	18,11
0,25	18,06	18,06	17,95	18 43	18 35	18,11
0,10	18,06	18,05	17,93	18 43	—	—

3. *Mercurijodid.*

Tabelle IX.

Aufbewahrungsdauer in Stdn	0	6	24	0	6	24
HgI_2 in g pro 1 l Saft	Rohrzucker in %			Reduzierender Zucker in %		
0	18,06	18 04	17,69	0,41	0,47	0,48
0,25	18,06	18,06	17,99	0,41	0,40	0,50
0,10	18,06	18,06	17 98	0 41	0,42	0,50
0,05	18 06	18,06	17,98	0 41	0,42	0,49

4. *Natriumbenzoat.*

Tabelle X.

Aufbewahrungsdauer in Stdn	0	6	24	0	6	24
Na-Benzoat in g pro 1 l Saft	Rohrzucker in %			Reduzierender Zucker in %		
0	20,10	20,06	19,86	0,25	0,28	0,34
5,0	20,10	20,02	19,99	0 25	0,27	0,32
3,0	20,10	20 04	20,03	0,25	0,27	0,31
2 0	20,10	20,06	20,04	0,25	0,26	0,31
1,0	20,10	20,10	20,04	0,25	0,28	0,32
0,5	20,10	20,08	20,02	0,25	0 28	0,31
0,1	20,10	20,08	19,92	0,25	0,29	0,24

5. *Natriumsalicylat.*

Tabelle XI.

Aufbewahrungsdauer in Stdn.	0	6	24	0	6	24
Na-Salicylat in g pro 1 *l* Saft	Rohrzucker in %			Reduzierender Zucker in %		
0	18,66	18,64	17,77	0,31	0,34	0,62
3,0	18,66	18,58	18,64	0,31	0,35	0,40
2,0	18,66	18,60	18,55	0,31	0,36	0,41
1,0	18,66	18,64	18,53	0,31	0,35	0,40
0,5	18,66	18,65	18,53	0,31	0,35	0,36
0,1	18,66	18,65	18,65	0,31	0,34	0,36

6. *β-Naphthol.*

Tabelle XII.

Aufbewahrungsdauer in Stdn.	0	6	24	Aufbewahrungsdauer in Stdn.	0	6	24
β-Naphthol in g pro 1 *l* Saft	Rohrzucker in %			β-Naphthol in g pro 1 *l* Saft	Rohrzucker in %		
0	16,85	16,85	15,97	1,0	16,85	16,85	16,81
3,0	16,85	16,85	16,80	0,5	16,85	16,85	16,81
2,0	16,85	16,85	16,81	0,1	16,85	16,85	16,21

7. *Borax.*

Tabelle XIII.

Aufbewahrungsdauer in Stdn.	0	6	24	0	6	24
Borax in g pro 1 *l* Saft	Rohrzucker in %			Reduzierender Zucker in %		
0	19,25	19,21	18,72	0,25	0,27	0,76
2,0	19,25	19,23	19,19	0,25	0,25	0,26
1,0	19,25	19,23	19,11	0,25	0,25	0,31
0,5	19,25	19,23	—	0,25	0,25	0,52

8. *Chloroform.*

Tabelle XIV.

Aufbewahrungsdauer in Stdn.	0	6	24	0	6	24
$CHCl_3$ in g pro 1 *l* Saft	Rohrzucker in %			Reduzierender Zucker in %		
0	19,25	19,21	18,72	0,25	0,27	0,76
1,0	19,25	19,25	19,19	0,25	0,26	0,32
0,5	19,25	19,23	19,02	0,25	0,26	0,32
0,2	19,25	19,23	—	0,25	0,27	—

Wir haben neben den Versuchen in den Tabellen V bis XIV noch zahlreiche diesbezugliche Versuche ausgefuhrt. Aus diesen Versuchen geht hervor, daß von den untersuchten Mitteln das Mercurichlorid die starkste antiseptische Wirkung besitzt. Formalin, Natriumbenzoat und Natriumsalicylat haben ungefahr gleiche faulniswidrige Wirksamkeit. Mercurijodid ist ein starkes Gift; es hat aber eine geringere antiseptische Wirkung als Mercurichlorid. Die Wirksamkeit von Borax und Chloroform als antiseptische Mittel ist geringer als diejenige der obenerwahnten Substanzen. β-Naphthol besitzt eine reduzierende Wirkung; deshalb ist der mit ihm versetzte Zuckerrohrsaft als Material fur die Zuckerbestimmung ungeeignet.

Neutrales und basisches Bleiacetat haben auch eine ziemlich starke antiseptische Wirkung aufzuweisen. Bei der Bestimmung des Zuckergehaltes des Zuckerrohrsaftes wird im allgemeinen der Saft mit diesen Reagenzien gereinigt. Wir haben in diesem Versuch zu 100 ccm Zuckerrohrsaft 2 g basischen Bleiacetats zugesetzt und nach bestimmten Zeiten mit 20 cm Rohr den Polarisationsgrad des Saftes ermittelt (Tabelle XV).

Tabelle XV.

Aufbewahrungsdauer in Stdn.	0	6	12	24	48
Polarisationsgrad	69,65	69,65	69,70	69,75	69,80

Wir haben dann zu 50 ccm Zuckerrohrsaft 10 ccm einer 10% igen Bleiacetatlosung hinzugefugt und nach verschiedenen Zeiten mit Natriumcarbonat entbleit. Die Veranderungen der Menge von reduzierendem Zucker im Saft sind in Tabelle XVI wiedergegeben.

Tabelle XVI.

Aufbewahrungsdauer in Stdn	Saft aus dem reifen Rohr	Saft aus dem überreifen Rohr
	Reduzierender Zucker in %	
0	0,34	0,66
24	0,36	0,70
48	0,38	0,72

Es wurde dann untersucht, ob sich durch Zusatz verschiedener antiseptischer Mittel die Wasserstoffionenkonzentration des Zuckerrohrsaftes verandert (Tabelle XVII).

Die Veranderungen der pH-Werte durch Zusatz der antiseptischen Mittel sind beim Zuckerrohrsaft bedeutend geringer als beim Wasser. Natriumbenzoat oder Natriumsalicylat aber uben auf den pH-Wert des Saftes keine Wirkung aus. Wenn dem Zuckerrohrsaft die anderen Mittel zugesetzt werden, so wird er fur die genaue pH-Bestimmung unbrauchbar.

Auf Grund der obenerwahnten Versuchsreihen haben wir die fur die voll

Tabelle XVII.

Zugesetztes Mittel in g pro 1 l Flüssigkeit	Gefundener pH-Wert		Zugesetztes Mittel in g pro 1 l Flüssigkeit	Gefundener pH-Wert	
	Zuckerrohrsaft (pH=5,6)	Wasser (pH=5,8)		Zuckerrohrsaft (pH=5,6)	Wasser (pH=5,8)
0,5 ccm Formalin	4,8	5,0	1 g Na-Salicylat	5,5	6,0
1 ccm Chloroform	5,3	5,0	2 g Borax	6,6	8,8
1 g Na-Benzoat	5,6	6,2	1 g β-Naphthol	5,3	5,5

ständige Konservierung des Zuckerrohrsaftes notwendigen Mengen der fäulniswidrigen Mittel festgestellt (Tabelle XVIII).

Tabelle XVIII.

Mercurichlorid	0,01%	Na-Salicylat	0,05%	Chloroform	0,1%
Formalin	0,05%	Na-Benzoat	0,05%	Borax	0,2%
Mercurijodid	0,05%	β-Naphthol	0,05%		

III. Veränderungen des Zuckergehaltes des Zuckerrohrsaftes durch Bestrahlung mit Sonnenlicht.

Das Zuckerrohr gedeiht bekanntlich nur in der tropischen oder subtropischen Zone sehr gut. Eine Untersuchung scheint daher von großem Interesse zu sein, wie sich die Zuckerarten im Zuckerrohr durch unmittelbare starke Sonnenstrahlen verändern.

In dieser Versuchsreihe haben wir zunächst die Differenz zwischen dem Zuckergehalt des Saftes aus der von Osten und der von Westen bestrahlten Seite ein und desselben im Feld stehenden Zuckerrohrstengels bestimmt. Die Zuckerbestimmung wurde nach der Methode von Hagedorn und Jensen (6) ausgeführt (Tabellen XIX und XX).

Tabelle XIX.

15 Monate altes Zuckerrohr.

Gezählt von unten	Saft aus der östlichen Seite			Saft aus der westlichen Seite		
	Grade Brix	Rohrzucker in %	Glucose in %	Grade Brix	Rohrzucker in %	Glucose in %
3. Knoten	20,6	17,9	1,30	19,8	16,6	1,57
6. "	19,7	17,0	1,90	20,1	16,6	1,93
9. "	20,6	16,8	2,27	20,5	17,6	2,12
12. "	20,9	17,7	2,56	21,2	17,2	2,38
15. "	22,3	20,5	1,31	23,0	20,5	1,15

Tabelle XX.

16 Monate altes Rohr.

Gezählt von unten	Saft aus der östlichen Seite			Saft aus der westlichen Seite		
	Grade Brix	Rohrzucker in %	Glucose in %	Grade Brix	Rohrzucker in %	Glucose in %
3 Knoten	19,2	18,3	1,65	20,2	18,0	1,22
6. "	22,3	20,9	0,41	21,7	19,7	0,62
9. "	22,2	19,6	0,46	21,9	20,7	0,37
12. "	21,1	18,3	0,67	21,1	18,9	0,81

Aus den oben angeführten und noch anderen Versuchen können wir schließen, daß zwischen dem Zuckergehalt der Ostseite des Rohrs und dem der Westseite kein Unterschied besteht.

Wir haben dann untersucht, ob zwischen der Zuckermenge im Saft aus der östlichen Seite eines im Feld stehenden Zuckerrohrs und derjenigen aus der nördlichen Seite desselben Rohrs irgendein Unterschied besteht (Tabellen XXI und XXII).

Tabelle XXI.

15 Monate altes Rohr.

Gezählt von unten	Saft aus der östlichen Seite			Saft aus der nördlichen Seite		
	Grade Brix	Rohrzucker in %	Glucose in %	Grade Brix	Rohrzucker in %	Glucose in %
3. Knoten	21,3	18,1	0,69	21,4	18,1	0,81
6. "	21,9	17,7	0,69	21,1	18,3	0,67
9 "	21,5	18,5	0,67	22,5	20,0	0,54
12. "	23,3	19,9	0,55	23,9	20,2	0,51
15 "	23,7	22,8	0,50	23,4	21,3	0,26

Tabelle XXII.

16 Monate altes Rohr.

Gezählt von unten	Saft aus der östlichen Seite			Saft aus der nördlichen Seite		
	Grade Brix	Rohrzucker in %	Glucose in %	Grade Brix	Rohrzucker in %	Glucose in %
3 Knoten	19,2	18,3	0,65	20,0	19,0	0,78
6. "	22,3	20,9	0,41	22,1	21,4	0,46
9. "	22,2	19,5	0,45	22,4	19,8	0,36
12. "	21,1	20,1	0,67	21,6	20,1	0,82
15. "	22,0	20,6	0,28	21,1	20,1	0,46

Wie aus Tabelle XXI und XXII ersichtlich, ist hierbei auch keine Differenz zu erkennen.

Es wurde ferner gepruft, welcher Unterschied zwischen dem Zuckergehalt des Saftes aus der nordlichen Seite eines Rohrstengels und demjenigen aus der sudlichen Seite desselben Stengels besteht (Tabelle XXIII und XXIV).

Tabelle XXIII.
13 Monate altes Rohr.

Gezählt von unten	Saft aus der nördlichen Seite			Saft aus der südlichen Seite		
	Grade Brix	Rohrzucker in %	Glucose in %	Grade Brix	Rohrzucker in %	Glucose in %
3 Knoten	19,8	19,0	0,36	19,3	17,9	0,47
6 〃	19,1	15,5	1,13	18,8	14,9	1,25
9 〃	20,2	19,0	0,41	19,8	18,5	0,55
12 〃	19,2	17,3	1,45	18,8	16,0	1,57

Tabelle XXIV.
18 Monate altes Rohr.

Gezählt von unten	Saft aus der nördlichen Seite			Saft aus der südlichen Seite		
	Grade Brix	Rohrzucker in %	Glucose in %	Grade Brix	Rohrzucker in %	Glucose in %
3 Knoten	18,6	16,1	0,07	18,6	15,9	0,07
6. 〃	21,4	18,5	0,06	20,4	18,5	0,07
9 〃	22,0	21,2	0,12	21,4	19,5	0,18
12 〃	23,4	19,9	0,04	22,0	19,3	0,06
15 〃	24,2	23,2	0,07	21,7	19,2	0,06

Tabelle XXIII und XXIV zeigen, daß der Brixgrad und Rohrzuckergehalt des Saftes aus dem schattigen Teil eines im Feld stehenden Zuckerrohrstengels etwas hoher sind, als diejenigen des Saftes aus dem Sonnenlicht empfangenden Teil, dass aber die Menge des reduzierenden Zuckers beim letztgenannten Saft großer ist, als beim erstgenannten.

Zusammenfassung.

1. Es wurden Versuche uber die Bestimmungsmethoden der Zucker im Zuckerrohrsaft mitgeteilt.
2. Es wurde der Einfluß verschiedener Reagenzien auf die Zersetzung der Zuckerarten im Zuckerrohrsaft untersucht.
3. Es wurde auch untersucht, welche Wirkung die Sonnenstrahlen auf den Zuckergehalt des Zuckerrohrs ausuben.

Literatur.

(1) T. Yoshida, Y. Iwata u. K. Yamafuji; Enzymol., 2, 342 (1938)
(2) K. Yamafuji u. T. Yoshida; Enzymol., im Druck
(3) K. Yamafuji u. T. Yoshida; Biochem. Zs., im Druck.
(4) R. Ofner, Zs. Zuckerind. cs. Rep., 53, 249 (1931).
(5) N. Schoorl, Handb. Pflanzenanlyse, Bd. II, Teil 1, S. 787.
(6) H. C. Hagedorn u. B. N. Jensen; Biochem. Zs., 135, 45 (1923).

Studies on the Enzyme in Young Mycelia of *Aspergillus Oryzae*.

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(Received Mar, 7, 1939)

INTRODUCTION.

There are many kinds of enzymes produced by *Aspergillus Oryzae*. There is no need of giving special references to literature on this subject as the fact is well known.(1~2) All of the literature makes references to enzymes in the full growth of the mycelia mass of this fungus. In this study, the author made experiments especially with young mycelia, the word “young mycelia” being used in the sense of fungus which has been cultivated but on which no spores have yet grown. Recently, especially in Japan, in the industrial use, i. e., in brewing Japanese saké and in manufacture of medicines, young mycelia of this fungus are very largely used. So the author undertook to study the enzymes in the young mycelia of this mold, and he will now describe 13 kinds of enzymes which he found in this experiment.

EXPERIMENT.

Materials and Enzyme Solution.

The materials used in this experiment were prepared in the following manner; the fungus was inoculated in a synthetic solution, and waiting until there was a sufficient growth of mycelia, they were taken off and washed with water, dried at a low temperature, and ground into powder. The enzymatic solution was prepared as follows:— one gram of the above mentioned fungus powder put into 100 cc of distilled water and left for four hours at a temperature of 40°C, the mixture being frequently stirred. The solution was then put through a Zeitz-filter, and the clear solution thus produced was used.

Results of the Experiment.

1. β-*h*-Fructosidase: This enzyme acted on one mol of saccharose and hydrolysed it into one mol of glucose and one mol of fructose. The following method was used to measure the enzyme action: 2% saccharose was used as substrate, 10 cc of it was put into a test-tube, and 5 cc of the enzyme solution was added. In order to keep the solution from putrifying 0.5 cc toluen was added. The mixtare was left in a thermostat at 40°C, for 24 hours. After which it was

put in into a boiling water bath to stop the action of the enzyme. All the fluid was then put into a graduated flask, water was added and the whole solution was increased to a volume of 50 cc, 20 cc of this was taken and the amount of reducing sugar was measured according to the BERTRAND method. From this value that of blank test was deducted and with the remaining number, the power of this enzyme was showen by remaining value.

Table 1.

a : 2% saccharose solution 10 cc + 1% enzyme solution 5 cc + toluen 0.5 cc.
b : 2% saccharose solution 10 cc + 1% boiled enzyme 5 cc + toluen 0.5 cc.
Incubation at 40°C for 24 hours.

Fxperiment number	0.5% $KMnO_4$ solution (cc)		
	a	b	a—b
1	4 00	0 35	3 65
2	4 00	0 40	3 60

As shown in table 1, there is a strong *β-h*-fructosidase in the young mycelia of *Aspergillus Oryzae.*

2. *α-d*-Glucosidase : This is an enzyme which hydrolyse *α*-maltose or *α*-glucoside. In general, there is maltase in this enzyme. A 2% maltose solution was used as a substrate to measure this enzyme. When the enzymatic action was finished, the results were obtained by the BERTRAND method.

Table 2.

a : 2% maltose solution 10 cc + 1% enzyme solution 5 cc + toluen 0.5 cc.
b : 2% maltose solution 10 cc + 1% boiled enzyme 5 cc + toluen 0.5 cc.
Incubation at 40°C for 24 hours.

Experiment number	0 5% $KMnO_4$ solution (cc)		
	a	b	a—b
1	10 95	9 30	1 65
2	10 95	9 20	1.75

As seen from the above table, *α-d*-glucosidase can be clearly demonstrated in this enzyme solution.

3. *β-d*-Galactosidase: This enzyme is also called lactase. It decomposes lactose into galactose and glucose. We used as a substrate a 2% lactose solution. As mentioned above, the enzymatic decomposition product was measured by the BERTRAND method with the following results:

Table 3.

a: 2% lactose solution 10 cc + 1% enzyme solution 5 cc + toluen 0.5 cc.

b: 2% lactose solution 10 cc + 1% boiled enzyme 5 cc + toluen 0.5 cc.

Incubation at 40°C for 24 hours.

Experiment number	0.5% $KMnO_4$ solution (cc)		
	a	b	a—b
1	10.30	9.00	1.30
2	10.30	9.00	1.30

As indicated in table 3, this enzyme solution contained very little *β-d*-glucosidase.

4. *β-d*-Glucosidase: There is emulsin, in general, in this enzyme. In this experiment, amygdalin was used as substrate.

Table 4.

a: 2% amygdalin solution 10 cc + 1% enzyme solution 5 cc + toluen 0.5 cc.

b: 2% amygdalin solution 10 cc + 1% boild enzyme 5 cc + toluen 0.5 cc.

Incubation at 40°C for 24 hours.

Experiment number	0.5% $KMnO_4$ solution (cc)		
	a	b	a—b
1	18.2	0	18.2
2	18.2	0	18.2

As noted in the above table, emulsin in this fungus enzyme worked very powerfully.

5. *α*-Amylase: In order to find out whether this enzyme was present or not, a starch solution was used, and the experiment was carried out through iodine reaction. 5 cc of this enzyme solution was added to 10 cc of 2% soluble starch solution, and was left at a temperature of 40°C for one hour. Then, iodine was added to the solution which gave it a red-brown colour, when a boiled solution of

this enzyme was used, and iodine added, it showed a blue colour. Thus we concluded that this fungus enzyme contained strong α-amylase enzyme.

6. β-Amylase: This enzyme is one which saccharifies starch. So we used 2% soluble starch as substrate and allowed this enzyme to act on it. The amount of sugar produced was measured by the BERTRAND method.

Table 5.

a: 2% soluble starch solution 10 cc + 1% enzyme solution 5 cc + toluen 0.5 cc.
b: 2% soluble starch solution 10 cc + 1% boiled enzyme 5 cc + toluen 0.5 cc.
Incubation at 40°C for 24 hours.

Experiment number	0 5% $KMnO_4$ solution (cc)		
	a	b	a—b
1	9.70	5.00	4.70
2	9.60	5 00	4.60

Table 5 shows that this enzyme solution showed strong β-amylase.

7. Papain type protease: This enzyme is one of the proteinase which has about pH 5.2 optimum hydrogen ion concentration. In our experiment, we used gelatin and casein as substrates on which we allowed this enzyme to work. Then we measured the amino acid produced by the SÖRENSEN method, with the following result:—

Table 6.

a: 2% gelatin solution (or 4% casein solution) 10 cc + 1% enzyme solution 5 cc + toluen 0.5 cc......pH 5.2.
b: 2% gelatin solution (or 4% casein solution) 10 cc + 1% boiled enzyme 5 cc + toluen 0.5 cc......pH 5.2.
Incubation at 40°C for hours.

Substrata	Experiment number	$N/10$ $Ba(OH)_2$ solution (cc)		
		a	b	a—b
Gelatin	1	0.70	0.45	0.25
〃	2	0.70	0.50	0.20
Casein	1	1.20	0.60	0.60
〃	2	1.25	0 60	0.65

In the above table, it can be clearly seen that in this enzyme solution, there is papain type proteinase.

8. Peptide decomposing enzyme: This peptide decomposing enzyme is one which decomposes peptone at about pH 8.0. Using peptone as a substrate of this enzyme, we measured the amino acid produced after the reaction, through the SÖRENSEN method.

Table 7.

a: 2% peptone solution 10 cc + 1% enzyme solution 5 cc + toluen 0.5 cc.

b: 2% peptone solution 10 cc + 1% boiled enzyme 5 cc + toluen 0.5 cc.

Incubation at 40°C for 24 hours.

Experiment number	$N/10$ $Ba(OH)_2$ solution (cc)		
	a	b	a—b
1	3 80	1 95	1 90
2	3 85	1 95	1 95

As shown in the above table, it can be easily seen that in this enzyme solution, there is peptide decomposing enzyme.

9. Lab: In order to test the lab enzyme, the following two methods were used.

1st experiment:— Using fat free milk, pasteurized.

Table 8.

a: Fat free milk 10 cc + 1% enzyme solution 5 cc + toluen 0.5 cc.

b: Fat free milk 10 cc + 1% boiled enzyme 5 cc + toluen 0.5 cc.

Incubation at 40°C for 24 hours.

Experiment number	a	b
1	coagulated	not coagulated
2	coagulated	not coagulated

2nd experiment:— Using casein solution.

1.5 g casein was dissolved in 25 cc of sodium bi phosphate solution, and was mixed with 25 cc of 0 4% calcium chloride solution, the mixture being used as substrate.

Table 9.

a: Casein solution 10 cc + 1% enzyme solution 5 cc + toluen 0.5 cc.

b: Casein solution 10 cc + 1% boiled enzyme 5 cc + toluen 0 5 cc.

Incubation at 40°C for 24 hours.

Experiment number	a	b
1	coagulated	not coagulated
2	coagulated	not coagulated

As shown by the experiment, in this enzyme solution, there was lab coagulating milk and casein.

10. Urease: In 10 cc of 2% urea solution was added 5 cc of this enzyme solution and 0.5 cc of toluen. This was kept, for a whole day, in a bath at a constant temperature of 40°C. When NESSLER reagent was added, it produced a

clear reddish brown colour. By this, we could see that there was urease in this enzyme solution. In addition we used as blank test, a boiled solution of this enzyme, but the reaction was negative.

11. Peroxidase: 5 cc of 1% pyrogallic acid was mixed with 5 cc of 3% hydrogen peroxide and to this was added 5 cc of this enzyme solution. When this was left in a room at a temperature of ca. 20°C, purpurgallin crystals were clearly seen. But when boiled enzyme were used as control test, the reaction was negative.

12. Indophenolase: To this fungus enzyme, a few drops of "Nadi" reagent were added. After 30 minutes, or an hour, a rose-red or red-violet colour appeared, but the blank test was negative.

13. Catalase: 1% hydrogen peroxide was used as substrate and was added to the enzyme solution. Using permanganate solution, the reaction was tested.

Table 10.

a: 1% hydrogen peroxide solution +10 cc enzyme solution 5 cc.
b: 1% hydrogen peroxide solution +10 cc boiled enzyme 5 cc.
Incubation at 40°C for 30 minutes.

Experiment number	0.5% $KMnO_4$ solution (cc)		
	a	b	a—b
1	1.90	0.30	1.60
2	1.90	0.30	1.60

As shown in the above table, this enzyme certainly has catalase action.

SUMMARY.

This study was made in order to test the enzymes which are produced by young mycelia of *Aspergillus Oryzae*. It was found that there were 13 kinds of enzymes, i. e, *β*-*h*-fructosidase (saccharase), *α*-*d*-glucosidase (maltase), *β*-*d*-galactosidase (lactase), *β*-*d*-glucosidase (emulsin), *α*-amylase, *β*-amylase, papain type protease, peptide decomposing enzyme, lab, urease, peroxidase, indophenolase and catalase.

Among these, peroxidese and indophenolase were weak.

A report will probably be made later of enzymes other than those noted above.

BIBLIOGRAPHY.

(1) Thom, C., and Church, M.: The Aspergilli, 59, (1926).
(2) Buchanan, R. E., and Fulmer, E. I.: Physiology and Biochemistry of Bacteria, III, 33 ~157 (1930).

Studies on Vitamin B_2 Complex.

III—Further Studies on the Growth-promoting Factor in Liver Extract.

By Ume TANGE.

From the Institute of Physical and Chemical Reserch.

(Received Oct, 20, 1939.)

In the previous paper,(1) dealing with the vitamin B_2 complex, I presented the evidence for the existence of growth-promoting factor (or factors) which could be separated from liver extract by treating its concentrated aqueous solution, after an exhaustive adsorption with acid clay, with large volumes of a mixture of alcohol and ether. This factor may be similar to or identical with factor W described by Frost and Elvehjem.(2)

In this paper I wish to present further proof that the profound growth-stimulating property of the alcohol-ether insoluble fraction is due to a factor distinct from riboflavin and to give some properties.

EXPERIMENTAL.

The basal ration described previously was slightly modified, and it has the following composition, supplemented daily with 1 drop of cod liver oil:

Purified fish protein	20%
Dextrinized corn starch	53
White corn	15
Butter fat	8
McCollum's salt	4

From the statement of Frost and Elvehjem,(2) we can assume that this ration supplies sufficient amounts of vitamins B_4 and B_5, but is low in flavin and factor W. If flavin were only deficiency in this ration, normal growth should result when flavin is added. No such result appeared as seen in Fig. 1 (b). On the other hand, the striking effect on the growth was obtained by supplementing the liver concentrate to flavin (Fig. 1 (c) and (d)). If the liver growth factor was alone added, there was good growth for some interval, but followed thereafter by decline. At this condition, if flavin was added, there was immediate resumption of growth. If both flavin and liver growth factor were added from the beginning to the diet, there was profound growth response. These results demonstrated very clearly that there is a remarkable supplementary action between flavin and liver growth factor in the nutrition of rats (Fig. 2 (b), (c) and (d)).

METHODS AND RESULTS.

Young rats weighing between 40 and 50 g were placed on the basal diet and each received daily 20 γ of vitamin B_1 hydrochloride. After being constant in weight for several days, the rats received the test doses of the different fractions supplemented by 20 γ of flavin daily. The control animals received flavin only. Three to six rats were used for each test and growth was observed for a period of six weeks. Some variations had been noted occasionally in the growth responses of rats from different litters, but within a litter, growth responses were fairly regular.

Flavin was prepared from dried egg-white powder extracting with a mixture of methanol-water (3 : 1), and concentrating the extracts under diminished pressure. The amounts of flavin in the concentrate thus obtained was estimated as lumiflavin by Pulfrich's photometer. This raw flavin was provided for rats at the level corresponding to 20 γ of pure flavin.

Preparation of the growth factor from liver extracts.

(a) Koehn and Elvehjem,(3) Macrae and Edgar(4) have reported that growth promoting factor for rats can be extracted from liver concentrate with amyl alcohol. The following procedure, therefore, was carried out: 300 cc of liver extract,(1) free from flavin (1 cc corresponding to 3 g of dry liver) previously treated with acid clay at pH 3.0, was adjusted to pH 1.4 with H_2SO_4 and extracted five times with 500 cc of amyl alcohol. The amyl alcohol solution was shaken three times with 400 cc of 10% NaOH. The alkali extracts were combined and, after neutralizing with HCl, the amyl alcohol was completely removed in vacuo. The effect, however, of the amyl alcohol extract on animal growth, as seen in Fig. 2 (a) and (a'), was not so good as that with acidified aqueous acetone extract obtained by the method described in the following: Two litres of the liver concentrate* (1 cc equivalent to 2 g of fresh beef liver), previously extracted with a mixture of methanol-water, acidified with H_2SO_4, and then treated with acid clay to remove flavin, having an acid reaction of a pale green-yellowish colour was treated twice with 100 g of acid clay each time to remove a trace of flavin which might remain in the solution, then the filtrate from acid clay adsorbates was neutralized with NaOH, and concentrated to about 200 cc in vacuo. Into the dark thick solution, 92% ethyl alcohol was added and the resulting precipitates were separated from the clear alcoholic solution. After similar treatment was repeated twice more, the combined alcoholic solution was evaporated. The 92% alcohol-soluble liver fraction was now placed in 300 cc of 0.2% HCI aq. solution and 2700 cc of acetone was added with constant stirring. The acetone fraction was decanted off and the residue was treated in the same way with a mixture of 600 cc of 0.2% HCl aq. solution and 900 cc of acetone. The similar procedure was repeated five times more. The acetone was recovered from the combined extracts by distillation. The resulting dark, aqueous solution was diluted with distilled water, considerable denatured protein separated out. This was filtered and the clear brown solution free from flavin was concentrated to about 200 cc for use and stored in the refrigerator.

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* The liver concentrate used as the starting material was kindly provided by Dr. F. Inukai, to whom I wish to express my great indebtedness.

Some experiments of the chemical properties of the growth-promoting factor.

1. Adsorption on charcoal — As I found that the acidified aqueous acetone filtrate contained the active principle, charcoal adsorption according to the method of Edgar et al.(5) was carried out: The solution was adjusted to pH 2.5 with HCl and treated with charcoal. The charcoal was washed with 0.1 *N* HCl and then eluted with 0.25 *N* NaOH, being allowed to stand overnight in the ice box. The elution was repeated once more. The eluate and filtrate were fed by separatory or combination at high levels; neither of them showed any appreciable growth-stimulating effect (Fig. 3 (a)).

2. Precipitation with acetone — The 92% alcohol-soluble fraction diluted with water of half volume of the solution was mixed with acetone. After standing overnight in the cold, the acetone solution was decanted from the gummy precipitates. After repeating similar treatment once more, the combined acetone was removed in vacuo. The greater part of activity was found in the soluble part, whilst the precipitates appeared inactive (Fig. 3 (b)).

3. Phosphotungstic acid precipitation — 60 cc of the 92% alcohol-soluble fraction diluted with water, acidified with H_2SO_4 to pH 1, and a 20% phosphotungstic acid solution was added until there was no further precipitate. After standing 24 hours with occasional stirring, the precipitates were filtered off. The filtrate, after removal of the excess phosphotungstic acid by the usual means, was found to have vitamin activity approximately equal to that of the starting alcohol-soluble fraction. The phosphotungstic precipitate was decomposed by H_2SO_4 and its filtrate was also tested, showing negative results (Fig. 3 (c)).

4. Barium precipitation — 100 cc of the acetone filtrate were diluted with an equal volume of distilled water and treated with a solution of 10 g of barium acetate in 25 cc of H_2O and the formed gummy precipitates were removed by filtering. The filtrate made alkaline to phenolphthalein with a cold, saturated barium hydroxide solution, and the slight precipitate was removed as before. The filtrate was then mixed with a 3-fold volume of alcohol and the formed flocculous precipitate centrifuged out. This precipitate was immediately dissolved in 50 cc of H_2O. After removal of barium quantitatively with sulphuric acid, the concentrate was stored in the ice-box. The alcoholic filtrate was

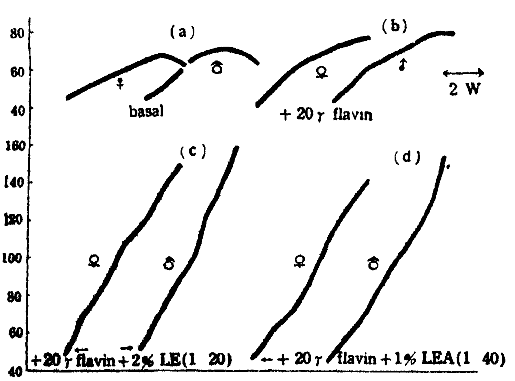


Fig. 1. Growth curves for rats on basal ration (a), basal ration + flavin (b), basal ration + LE (c) and basal ration + LEA (d). LE and LEA are liver concentrate, free from flavin, and 92% alcohol-soluble liver extract, respectively. (1 : 20) and (1 : 40), show that 1 part each of the preparation corresponds to 20 and 40 parts of fresh liver,

also freed of barium with sulphuric acid and prepared for assay. The feeding results of these fractions singly and together were given in Fig. 3 (d).

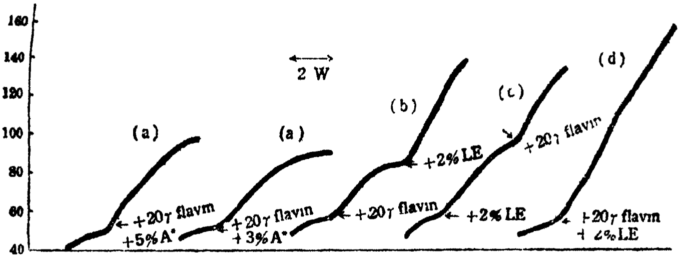


Fig. 2. Growth curves of rats receiving flavin alone, liver concentrate free from flavin, the combination of the two, and flavin + amyl alcohol extract of liver = A* (1 cc of A* is equivalent to 3 g of dry liver).

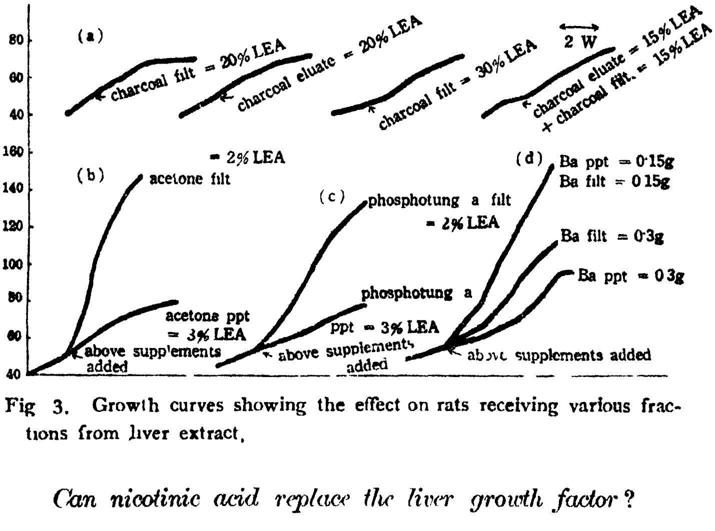


Fig. 3. Growth curves showing the effect on rats receiving various fractions from liver extract.

Can nicotinic acid replace the liver growth factor?

Recently the importance of nicotinic acid and its amide in the biological process is recognized, and there is some evidence that the acid or its amide has a growth-promoting action for rats or pigeons on various diets deficient in some factors of the vitamin B complex, while the others claim that these pyridine compounds have no significant effect on the growth of rats receiving vitamin B_1 and flavin as source of vitamin B complex. It is interesting, therefore, to find if nicotinic acid can replace the growth factor in liver extract. The experiment was carried out as follows: The rats received the basal diet supplemented with vitamin B_1. When they ceased to grow, the rats were divided into two groups. One group of the rats received 20 γ of

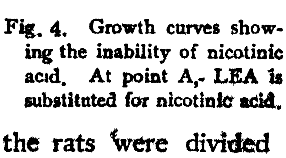


Fig. 4. Growth curves showing the inability of nicotinic acid. At point A, LEA is substituted for nicotinic acid.

flavin and 2 mg of nicotinic acid daily, while the other group was given, in addition to 20 γ of flavin daily, 2% of the 92% alcohol-soluble liver extract. The results in Fig. 4 showed that nicotinic acid had no significant increase in the growth rate and the acid could not be substituted for the growth factor found in the liver extract.

Discussion.

The observations described above confirm the previous finding(1) that in addition to vitamins B_1, B_4(?), B_6 and flavin, the rat requires still another factor (factor W?) to attain the maximum growth. This factor can be prepared from liver concentrate by dissolving in 92 per cent ethyl alcohol and treating the alcohol-soluble fraction with acidified aqueous acetone. The active factor is not adsorbed by acid earth at pH 1.4 and 3.0 or by charcoal at pH 2.5. The factor is readily soluble in ethyl alcohol and partially precipitated by barium salt, but not by phosphotungstic acid. Recently Edgar and Macrae(5)(6) described that the yeast-extract filtrate factor for rat is probably acidic, since it is precipitated by barium hydroxide in 90 per cent alcohol. In a recent paper Frost and Elvehjem(2) reported evidence suggesting a relation of factor W to pyridine nucleotides.

The comparison of the properties of the fraction, as already stated, in an aqueous acetone solution indicates that the factor promoting growth in rats is probably identical with factor W described by Frost and Elvehjem.(2)

The biological importance of the pyridine compounds, was recently recognized by the demonstration of Warburg and Eular that nicotinamide is the active group in certain coenzymes. Funk and Funk,(7) Frost and Elvehjem(2) showed that nicotinamide, and nicotinic acid and adenine nucleotides have growth-promoting action for pigeons and rats on certain rations. I have found, however, that nicotinic acid has no stimutating effect on the growth of rats, in agreement with the results of Edgar and Macrae,(4) who showed that the pyridine compounds cannot replace their yeast-filtrate factor or yeast-eluate factor, although they have isolated nicotinamide from the yeast-eluate fraction. Moreover, these workers demonstrated the presence of a growth factor for rats distinct from nicotinic acid in an amyl alcohol extract of liver and described that this factor may be identical with the yeast-filtrate factor. Dann and Subbaraw(8) reported that nicotinic acid does not cure rat dermatitis or prevent chick dermatitis and concluded that vitamin B_6 and the filtrate factor are both distinct from nicotinic acid. Recently Elvehjem and his coworkers claimed that a factor was isolated from liver and identified as nicotinic acid amide.(9) More recently Elvehjem and his colleagues(10) reported that nicotinic acid or its amide has no curative power on chick dermatitis and that liver extract contains a factor necessary for the prevention of a typical dermatitis in chick which is separate and distinct from vitamin B_1, vitamin B_6, flavin, nicotinic acid, and factor W.

Regarding the nature of the growth factor (factor W?), I wish to cite the claim of Frost and Elvehjem(2) on the relationship between factor W and pyridine nucleotides and on the chemical behaviour of the factor towards mercury and barium salts which indicated a similarity to the coenzymes. We must consider, however, the comment of Peters(11) who stated: "at present results on factor W are difficult to reconcile with other finding owing to the non-synthetic nature of the diet. Vitamins B_4 and B_6 were provided in the form of white corn composing 12 per cent of the diet which, it is stated by Frost and Elvehjem, also contain small yet adequate amount of the filtrate factor". Further experiments are, therefore, needed with regard to the nature of the growth-stimulating factor in liver.

SUMMARY.

1. A method for separation and concentration from liver extract of the growth-promoting factor (or factors) distinct from flavin is given.

2. The factor (or factors) is readily soluble in ethyl alcohol, and the addition of a large volume of acetone to the aqueous solution cannot precipitate the active factor. The factor is not precipitated by phosphotungstic acid, but partially by barium. It is not adsorbed by acid clay or charcoal at pH 1.4 and 2.5.

3. Normal growth is induced in rats when both flavin and liver growth factor are added to the basal ration.

4. Nicotinic acid cannot replace the active factor, as tested on growth of rats.

5. Comparison of the properties of the factor indicates that it is distinct from riboflavin, but may perhaps be identical with factor W described by Frost and Elvehjem.

I wish to thank Dr. U. Suzuki for his continued interest and advice concerning this work. I am also indebted to Miss T. Kaneko for her generous assistance in the preparation of the materials and the feeding of the animals.

(1) Ume Tange: Rikwagaku-kenkyu-jo Iho, **16**, 1058 (1937)
(2) D. V. Frost and C. A. Elvehjem: J. Biol., Chem., **121**, 255 (1937)
(3) C. J. Koehn and C. A. Elvehjem. J. Biol. Chem., **118**, 693 (1937)
(4) T F. Macrae and C. E. Edgar: Biochem. J., **31**, 2225 (1931)
(5) C. E. Edgar and T. F. Macrae. Biochem. J, **31**, 893 (1937)
(6) C. E. Edgar and T. F. Macrae: Biochem. J., **31**, 886 (1937)
(7) C. Funk and I. C Funk: J. Biol. Chem, **119**, XXXV (1937)
(8) W. J. Dann and Y. Subbaraw. J. Nutrition, **16**, 183 (1938)
(9) C. A. Elvehjem, R. J. Madden, F. M. Strong, and D. W. Woolley: J. Biol. Chem., **123**, 137 (1938)
(10) O. Mickelsen, H A. Waisman, and C. A. Elvehjem. J. Biol. Chem, **142**, 313 (1938)
(11) Annual Review of Biochemistry, **VII**, 317 (1938)

ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

Besearches on Mechanical Wood Pulp. Part I.

Chemical Analysis of Pinus densiflora, S. et. Z. and Pinus Thunbergii, Parl.

(pp. 305~308)

By Mamoru WATANABE.

(Agricultural Chemical Institute, Kyoto Imperial University Received March. 9, 1939.)

On the Relation between the Nitrogen Compounds of Seeds of Various Crops and the Diastatic Power of their Malts. Part IV.

Studies on Barley and Naked-barley.

(pp. 309~316)

By Hideo KATAGIRI and Narataro MUGIBAYASI.

(Agr. Chemical Laboratory, Kyoto Imperial University; Received Feb. 22, 1939.)

Experiments were carried out with the seeds of 23 species of barley and of 7 species of naked barley.

During the germination, hordein or glutelin nitrogen of all the seeds decreased and soluble nitrogen always increased, although the rate of decomposition of hordein or glutelin nitrogen and the rate of formation of the individual components of soluble nitrogen varied with the species of the seeds.

The activity of maltase in the germinated seeds was found to be quite different according to the species of the seeds.

It was concluded that the saccharifying power of the germinated seeds could be estimated from the amount of albumin nitrogen of ungerminated seeds including barley and naked barley, as was already suggested with the seeds of kaoliang, mung beans and rice (see Parts I, II and III).

Über den Stickstoff- und Aschengehalt der Kaninchenmuskeln.

(ss. 317～322)

Von M. Kandatsu.

(Aus dem Agrikulturchem Lab d Kaiserl Univers. Tokio, Japan
Eingeg. am 22, März, 1939)

On the Retting of Vegetable Fibre Materials.

(pp. 323～330)

By Toshio Nakahama.

(Yamashina Chemical Institute of Kanebo Silk Factory, Received Mar 8, 1939)

Part IX— The Selection of Useful Aerobes for the Bacterial Retting of Flax. It was found that an aerobic process was more effective than the natural retting of flax, and ten strains of aerobic bacteria were isolated from the aerobic retting vat.

One strain of bacilli (No. 5) was found to be most effective for the retting of flax, when pure fermentations of flax were carried out with all the bacteria isolated.

Part X— The Characteristics of the Useful Bacteria for the Aerobic Retting of Flax. The useful retting bacteria was found to reveal very much the same natures as *Achromobacter venosum*: spore was not found in the bacterial cells, milk was not changed, gelatin was not liquefied, nitrate was not reduced, indole was not produced by the bacteria, and the cells were motile, possessing long peritrichous flagella. It was, therefore, concluded that the bacteria belong to a kind of *Achromobacter venosum.*

The Bacteriocidal Quality of Sterilized and Non-sterilized Korean Condiments Against Dysentery Bacilli Group.

(pp. 331～336)

By Y. L. Pak. M. D.

Keijo, Chosen. (Seoul, Korea)
Received Feb 27, 1939.

The kinds of condiments used by Koreans for their daily food are numerous and are rich in taste of hot pepper, salt, and sourness and are used rather in larger quantity when compared with other countries. In consideration of a view that the daily use of such condiments may, possibly, give some favorable influence against various gastro-intestinal diseases, especially against the causative organism of acute infectious disease of the gut, the author had undertaken a study of various condiments which are in common use in Korea as to their sterility and durability over the dysentery bacilli group.

A series of various condiments of about 20 kinds were examined and they are; *Garlic* (Allium scorodoprasum), *black pepper* (Piper nigrum), *hot pepper* (Capsicum annum), *radish* (Raphanus sativus), *ginger* (Zingiber officinale), *mustard* (Brassica cerna), *rock mushroom* (Gyrophova esculent), *Digenea*, peel of Korean citron (Citrus aurantium), *onion* (Allium fistulosum), *mush-room* (Cortineluss shiitake), *soy* (salted soy bean juice), *miso* (soy bean paste), *hot-peppered miso*, *vinegar*, *vinegar flavored with citron*, *honey*, *sesame oil* (Oleum sessamin indicum), roasted sesame seed and *Korean molasses*, etc. These were divided into two groups; to one group after sterilizing and to the other without sterilizing were added dysentery bacilli of various strains; namely, Shiga, Komagome A, and B. After 30 minutes to 20 days the result was examined by determining the bacteriocidal action in the first group and in the second group, where no sterilization was made, the process reaching the point of sterility and also the phenomena of antagonism with various non pathogenic bacteria. The summary of the result is as follows;

1. Generally speaking, the condiments of the non-sterilized group possess stronger bacteriocidal power than those sterilized.

2. Black pepper, mustard, and vinegar are found to be most powerful in bacteriocidal action and honey, sesame oil, and rock mush room which were considered to be less bacteriocidal are also found to be almost equal in their strengths.

3. The condiments found least bacteriocidal are miso, (soy bean paste) mush room, radish and hot-pepper.

4. The dysentery bacilli of Komagome A strain showed weaker resistence to the condiments, whether sterilized or unsterilized, and Shiga and Komagome B strains are about equal in their resistance.

5. The condiments that had been sterilized showed lowered H ion concentration, and at the same time when the sourness and hot peppery taste had become weakened their bacteriocidal quality had, also, become weakened due to some probable change that might have occurred in their volatile substances.

6. Non-pathogenic bacteria attached to the condiments are stronger in resistance over the pathogenic bacteria and they are considered to be useful because of their antagonism against pathogenic organism within the gut.

7. The bacteriocidal property was due to the chemical constituents and hydrogen ion concentration of the condiments. Concerning the former property a further study is needed to ascertain its exact nature.

8. It was made clear in our experiment that mushroom took less than one-half hour to produce sterilization of dysentery bacilli of Komagome A strain, sesame oil about the same time for A and B strains, and Korean molasses the same for A strain, each manifesting its selective action. However, the soy took

2 hours for dysentery bacilli of Komagome B strain, and the peppered miso the same time for Komagome B strain, this also through their selective bacteriocidal actions.

(Author's abstract)

Eine Experimentelle Fabrikation von Geraucherten Hammelfleisch.

(ss. 337~347)

Von Dr. R. Sasaki, M. Kandatsu u. S. Minato.

(Aus dem Agrikulturchemischen Lab. d. Kaiserl. Univers. Tokio, Japan:
Eingeg. am 3, März, 1939.)

Carbohydrates of the Bulbs of Allium. IX.

Structure of Scorodose.

(pp. 348~352)

By Yoshijiro Kihara.

(Agricultural Chemical Laboratory, Tokyo Imperial University Received Mar. 22, 1939.)

Abbau der Aminosäuren durch Aspergillus Oryzae. III. Mitteilung.

(ss. 353~358)

Von T. Uyemura.

(Aus der Agrikult. Chem. Labor. Kaiserl. Univ. zu Tokio.)

α-Ketosauren wurden als Abbauprodukte von α-Aminosauren durch einige zum Aspergillus Oryzae gehorende Stamme unter Vergarungsbedingungen gebildet; α-Ketoisocapronsaure aus *l*-Leucin, α-Ketoglutarsaure aus *l*(+)-Glutaminsaure und *p*-Oxyphenylbrenztraubensaure aus *l*-Tyrosin.

1. Abbau von *l*-Leucin.

Zusammensetzung der Nahrlosung: Glykose 1.0 g, KH_2PO_4 0.3 g, $MgSO_4$ 0.01 g, $FeSO_4 \cdot 7H_2O$ Spur, *l*-Leucin 0.8 g, dest. Wasser 100 ccm (unter Zusatz von 2%ig. Kreide.) Die gesamte Menge von *l*-Leucin: 34 g.

17 Kolben (250 oder 300 ccm Nahrlosung enthaltend) werden geimpft und bei 31°C 26 Tage lang aufbewahrt.

Gewicht der Pilzdecke (nach 26 tagiger Kulturdauer) 13.2226 g,

N-Gehalt der Pilzdecke 3.89%.

α-Ketoisocapronsaure wurde aus atherischem Auszug von konzentrierter Vergarlosung isoliert. Ausbeute 1.2205 g.

Phenylhydrazon der α-Ketoisocapronsaure. Gelbe Nadeln aus Äther-Ligroin. Fp = 132~3°C. (Unkorr.)

Analyse;	N (%)	C (%)	H (%)
Gef.	12.77	65.35	7.09
Theo.	12.72	65.42	7.33

Oxim der α-Ketoisocapronsaure. Weisse Tafeln. Fp = 149°C. (Unkorr.)

Analyse;	N (%)	C (%)	H (%)
Gef.	9.46	50.04	7.18
Theo.	9.65	49.62	7.65

Aus *l*-Leucin wurde noch *d*-Leucinsaure (Ausbeute 0.7782 g) isoliert.

2. Abbau von *l*(+)-Glutaminsaure.

10 g von *l*(+)-Glutaminsaure werden im Wasser suspendiert, mit NaOH neutralisiert und zur Losung gebracht, dann mit 1000 ccm der unten beschriebenen zusammengesetzten Nahrlosung gemischt. Jeder Kolben (insgesamt 5 Kolben), 300 ccm der gemischten Losung enthaltend, wird geimpft und bei 31°~2°C 15 Tage aufbewahrt.

Zusammensetzung der Nahrlosung: Glykose 15 g, $Na_2HPO_4 \cdot 12H_2O$ 1.5 g, KH_2PO_4 2.0 g, $MgSO_4$ 0.1 g, $FeSO_4 \cdot 7H_2O$ Spur, dest. Wasser 1000 ccm.

pH des Gemisches (nach Sterilisation) 6.0,
pH der Vergarlosung 7.3,
Gewicht der Pilzdecke (nach 15 tagiger Kulturdauer) 3.5348 g,
N-Gehalt der Pilzdecke 5.16%.

Die Vergarlosung wurde zur Analyse verwandt, und es wurden weisse Kristalle aus dem atherischen Auszug in Wasser ausgelost. In salzsaurer Losung wurde mit 2·4-Dinitrophenylhydrazin HCl-Losung ein gelber Niederschlag von 2·4-Dinitrophenylhydrazon der -Ketoglutarsaure erhalten.

2·4-Dinitrophenylhydrazon der α-Ketoglutarsaure, Gelblich farbende Nadeln. Fp = 221°C.

Analyse;	N (%)	C (%)	H (%)
Gef.	17.40	40.98	3.29
Theo.	17.18	40.49	3.07

3. Abbau von *l*-Tyrosin.

3000 ccm zum Versuch gebrachte Nahrlosung wurde in folgenderweise zusammengesetzt und in 10 Kolben eingeteilt. (Die gesamte Menge von *l*-Tyrosin: 5.0 g) Glykose 1.50 g, $Na_2HPO_4 \cdot 12H_2O$ 0.10 g, KH_2PO_4 0.20 g, $MgSO_4$ 0.01 g, $FeSO_4 \cdot 7H_2O$ Spur, *l*-Tyrosin 0.50 g, dest. Wasser 100 ccm.

pH der Nährlosung (nach Sterilisation) 5.75,
pH der Vergarlosung (nach 15 tagiger Kulturdauer) 3.90,
Gewicht der Pilzdecke (wie oben) 4.7295 g,
N-Gehalt der Pilzdecke 3.89%.

p-Oxyphenylbrenztraubensaure wurde in weissen Kristallen aus dem atherischen Auszug isoliert, und dann in 2·4-Dinitrophenylhydrazon herausgesetzt. Ausbeute = 28.9 mg.

2·4-Dinitrophenylhydrazon der *p*-Oxyphenylbrenztraubensaure; Rotlich braunliche Nadeln aus Äther-Ligroin, Fp = 198°C. (Unkorr.)

Analyse;	N (%)	C (%)	H (%)
Gef	15 05	49 05	3.10
Theo.	15 55	49 98	3 32

Aus der Vergarlosung von *l*-Tyrosin bildet sich Acetaldehyd in ziemlich reichlicher Menge.

Studies upon the Moulds, Growing in High Concentration of Sulphuric Acid and Copper Sulfate.

(pp. 359～369)

By Tatsuzo SATO.

(Agricultural Chemical Laboratory, Tokyo Imperial University, Received Feb 27, 1939)

It is generally believed that most fungi are sensitive even to low concentrations of sulphuric acid and of copper sulfate.

The author has recently obtained four strains of moulds, which grow well in high density of sulphuric acid and copper sulfate.

Three of them, (*Oospora, Dematium, Penicillium*) are isolated from sulphuric acid solution, containing copper sulfate, which is employed in certain manufactory, and one of them (*Penicillium*) is isolated from copper sulfate solution of Bertrand's reagents.

The properties of the moulds are as follows

Oospora viscosa nov. sp.

Growth restricted on any culture media except on koji- and malt-extract agar. Colonies on koji extract agar, smooth, with wax like gloss, deeply wrinkled up and gloss lost when old, cream buff to avellaneous in color, (Ridgway's Color Standard), 8 mm deep, with mycelial bundle (1～2 mm). Young mycelium 45～85 μ by 1.5～1.8 μ, chlamydospore 6～9.5 μ by 2.4～4.5 μ. Dwarfish conidiophore not branched, 10～15 μ by 1～1.5 μ, sterigmata not observed. Conidia ovoid, sausage- or cylinder-shaped, 2.5～3 μ by 1～1.5 μ, aspect smooth, contents slightly granular. No variation on morphological properties in 7% sulphuric acid.

Opt. tem. for growth 28°C, and the inhibition below 14°C or above 37°C, opt. Ph 5.4. The fungus can grow vegetatively in the following concentrations of acids and copper sulfate: 0.05% $CuSO_4$, 7% H_2SO_4, 2.5% HCl, 2% HNO_3; killed in 40% sulphuric acid in 30 minutes, or in 35% sulphuric acid in 60 minutes. No fermentation on carbohydrates.

Dematium pullulans de Bary var. *acidiphylum* nov. var.

Fertile growth only upon koji extract- and malt extract-agar. Colonies on koji extract agar, smooth, very closely felted, 10 mm deep, with wax like gloss, then aerial hyphae produced and gloss lost with age, black to celandien green, finally hair brown. Young mycelium 45×2∼60×2.5 μ. Chlamydospore (6×5∼9×8 μ) chamois in color. Sometimes grows yeast-like cell (3×2.5∼4×3.5 μ). Conidipohore grows from mycelium alone, (5×1∼6×1.5 μ), conidia globose or elliptical (1×1.5∼1.5×2 μ). No change in morphological properties in 4% sulphuric acid.

Opt. temp. for growth 30°C, and no growth below 14°C or above 37°C. Opt. Ph 5.4. The fungus is able to grow vegetatively in the following concentrations of acids and copper sulfate: 0.1% $CuSO_4$, 5% H_2SO_4, 1% HCl, and killed in 15% sulphuric acid in 30 minutes, or in 10% sulphuric acid in 60 minutes. No fermentation on carbohydrates.

Penicillium cuprophylum nov. sp.

Colonies on Czapeck's soln. agar in the scarcely zonate group, floccose with marginal area of a deep floccose mass of mycelium, 6 mm deep and 5.4∼5 cm in diameter after 10 days; central areas greenish glaucous with ripening conidia, then turn to pale olive buff, and finally to light mineral gray, with reverse ochraceus-orange. Drops abundant, colorless, on koji extract agar orange yellow. Odor none. Gelatin not liquefied.

Conidiophores 20∼200 μ by 2∼3 μ, smooth walled and asymmetrically branched; branches 15∼20 μ long; metulae 12∼17×2∼3 μ, 2∼3 in the group: sterigmata about 7∼8 μ by 1∼1.5 μ, in verticils of about 1 to 5: conidia globose or elliptical 2∼3×1.5∼2.5 μ, smooth; conidial chains tangled. Hyphae 3 μ in diameter, smooth; in the presence of copper sulfate the older hyphae show a great distortion.

Opt. tem. for growth 28°C, and the inhibition below 0°C or above 37°C, opt. Ph 7.6 or 4.4. Grows vegetatively but does not sporulate in 19% copper sulfate solution. No fermentation on carbohydrates.

Penicillium biforme Thom var. *vitriolum* nov. var.

Colonies on Czapeck's soln. agar 7 mm deep, 7 cm in diameter in 10 days, gelatin not liquefied, floccose, zonate, first white, then central areas dark bluish glaucous with ripening conidia, changing to olive-buff, vinaceous-buff finally to light grayish olive. Reverse warm buff. Drops abundant. Odor none.

Conidiophore with smooth, walls about 20∼140 μ by 2∼3 μ, asymmetrically branched: branches 15∼25 μ long, metulae 12∼20×2∼3 μ, 2∼3 in the group; sterigmata about 10∼12 μ by 2.5∼3 μ, in verticils of about 1∼5: conidia globose or elliptical 2∼4×1.5∼3.5 μ, smooth. Conidial chain tangled. Hyphae 3 μ in diameter, smooth walled; in the presence of 21% copper sulfate the older hyphae show a great distortion.

Opt. tem. for growth 28°C, and the inhibition below 0°C or above 37°C. Opt. Ph 4.4. Grows vegetatively but does not sporulate in 21% copper sulfate solution. No fermentation on carbohydrates.

On a New Polypeptide Isolated from Eisenia Bicyclis. (Part I.)

The Isolation of "Eisenin" and its Hydrolysis Products.

(pp. 370∼376)

By Tosihiko OOHIRA.

(Agricultural Chemical Laboratory, Tokyo Imperial University Received; Apr. 4, 1939.)

A new polypeptide, named "Eisenin", $C_{13}H_{20}O_6N_4$, which gives intensive biuret reaction, has been isolated from the brown alga, Eisenia bicyclis (Kjellm.) Setchell. It crystallises from dilute alcohol in colourless long needles with silky luster and melts at 225∼226° (decomp.), sintering at about 180°. Eisenin is easily soluble in water and difficultly in absolute alcohol and acetone, but insoluble in ether, ethylacetate and other usual organic solvents. Eisenin is laevorotatory, $[\alpha]_D^{14°} = -54.3$, in aqueous solution. It gives an acid reaction and tastes sour. Eisenin contains one free carboxyl group which is determined by titration with normal alkaline hydroxide solution and also by preparation of *p*-bromphenacylester.

On hydrolysis with 25% sulphuric acid, "Eisenin" yields *d*-glutamic acid (2 mols), *d*-alanine (1 mol) and ammonia (1 mol), as follows:

$$C_{13}H_{20}O_6N_4 + 4H_2O = 2C_5H_9O_4N + C_3H_7O_2N + NH_3$$

The ammonium rest is readily detachable by heating it in weak alkaline solution. The original substance is not ammonium salt, since it gives no Nessler's reaction.

The author suggests, therefore, that the ammonia is formed as an acid amide.

The combination of these components in the polypeptide is now under investigation.

The following method was adopted for the isolation of "Eisenin" from the brown alga.

One kilo of the air dried and powdered alga was extracted three times with boiling 90∼95% alcohol, using 2 liters at a time. A large quantity of mannitol which had separated out on cooling from the united extract was filtered off and the filtrate was evaporated to a small volume (300∼400 cc) under diminished pressure and filtered again from the precipitate formed on evaporation. The filtrate was then evaporated to syrup and extracted with 500 cc of warm water. The solution was kept overnight in a refrigerator and freed of any precipitate that may have formed. The brownish clear solution thus obtained was purified as usual with basic lead acetate by which "Eisenin" was not precipitated. To the purified liquid, after concentration in vacuo to 300 cc, was added saturated aqueous mercuric acetate solution until the yellow precipitate began to occur, keeping the solution always nearly neutral with the addition of 10% aqueous sodium hydroxide solution. An equal volume of 90∼95% ethanol was then added to it and allowed to stand for several hours, whereby the yield of the precipitate substantially increased. The precipitate was collected and decomposed with hydrogen sulphide.

The filtrate from the mercuric sulphide was evaporated under reduced pressure to a small bulk and strong ethanol acidified with sulphuric acid was added to it with constant stirring, whereupon a large amount of crystals separated out. The crystals were collected, washed with ethanol and recrystallized from 80~90% ethanol, yield 8 g.

In an additional experiment with fresh alga, the author was able to isolate the same substance: showing that the polypeptide was not a degradation product formed during the drying of the alga.

The Influence of Monochromatic Lights on the Action of Enzymes.

(pp. 377~384)

By Reitaro Murakami.

(Agricultural College, Utunomiya; Received Jan. 31, 1939.)

[Report XIV.] Especially on the Influence of Ultra-violet Rays on the Action of Enzymes.

(Part 3.) Yeast Proteinase.

The influence of ultra-violet rays on the action of the proteinase in the yeast was investigated by using "Vim Ray" blue lamp. Enzyme solution was prepared as follows: After the liquefaction was completed by the addition of 25 cc of ethyl acetate into 250 g of "Oriental" pressed yeast, 500 cc of water was added. An acid produced during 2 hours was neutralized continuously by the addition of 10% ammonia water and then an ineffective solution for the tryptic and ereptic action was separated and washed by 1 liter of water. Then the yeast was suspende in 500 cc of water containing toluene and allowed to autolyze at room temperatur for 24 hours. An acetic acid solution was added to the autolyzate obtained b the filtration and after the pH of the liquid was converted to 5.0, 5 cc of $N/1$ acetate buffer of pH = 5.0 and 20 cc of alumina cream were added. The proteinase was obtained as a clear solution by the elution of the adsorbate with 44 c of 5% secondary ammoninm phosphate.

The author added 1 cc of the enzyme solution into the test tubes containin 5 cc of 5% gelatin and 5 cc of citrate buffer, the resulting pH of the solutio being 5.1.

The test tubes were placed within tin boxes, with filters at the front window and were incubated at 40°C and lighted by "Vim Ray" blue lamp as in th author's previous paper. [Report XII.]

The test tubes were taken out from each box at the intervals of 24, 48 an 72 hours after the incubation and then the end product of the hydrolysis was determined by the former method.

In this experiment, the action of the yeast proteinase was found to be promoted by the visible rays and ultra-violet rays, the latter producing a greater promotion than the former.

[Report XV.] Especially on the Influence of Ultra-violet Rays on the Action of Enzymes.

(Part 4.) Yeast Lipase.

The author has employed "Vim Ray" blue lamp to investigate the influence of ultra-violet rays on the action of the lipase in the yeast. Enzyme solution was prepared by the autolysis for 2 days at room temperature and then for overnight in an ice box after adding 10 cc of toluene and 500 cc of water into 200 g of "Oriental" pressed yeast.

The author added 5 cc of the enzyme solution into the test tubes containing 5 cc of castor oil, which was neutralized with $N/20$ NaOH solution and then treated as in the previous paper. [Report XII.]

The tubes were taken out from each box at the intervals of 8, 16 and 24 hours after the incubation and then the end products, fatty acids of the hydrolysis, were titrated using $N/20$ NaOH solution by Knitz's method.

In this experiment, the action of the yeast lipase was found to be promoted under iufluence of the visible rays and the ultra-violet rays, but the promotion was greater under the latter than the former.

[Report XVI.] Especially on the Influence of Ultra-violet Rays on the Action of Enzymes.

(Part 5.) Yeast Catalase.

"Vim Ray' blue lamp was used in order to investigate the influence of ultra-violet rays on the action of the catalase in the yeast. Enzyme solution was prepared an follows: After the liquefaction was completed by the addition of 50 cc of toluene into 150 g of "Oriental" yeast within one hour at 40°C, 200 cc of water was added and allowed to autolyze during overnight in an ice box. To the autolyzate obtained by the filtration was added 44 cc of $N/10$ HCl solution and then the total volume of the liquid made to 2000 cc with water and finally 150 cc of alumina cream was added. The catalase was obtained as a clear solution by the elution of the adsorbate with 400 cc of $M/30$ phosphate solution, with $pH = 7.6$.

The author added 1 cc of the enzyme solution into the test tubes containing 20 cc of $N/20$ H_2O_2 buffered with phosphate mixture (pH of the solution was 6.7) and it was treated as in the previous paper. [Report XII.]

The tubes were taken out from each box at the intervals of 5, 10 and 15 minutes after the incubation and the undecomposed hydrogen peroxide in the solution was determined by the titration with $N/20$ $KMnO_4$ solution.

In this experiment, the action of the yeast catalase was found to be inhibited by ultra-violet rays and promoted by visible rays. The action of the enzyme was also promoted under the rays containing both ultra-violet and visible, but the promotion for the enzyme was weaker than by the visible rays alone.

Studies on the Utilization and Digestion by Silkworms of the Carbohydrates contained in Mulberry Leaves.

(pp. 385～393)

By K. Kato.

(The Sericultural Experiment Institute of Gifu Prefecture; Received Mar. 8, 1939.)

Part VII. The change in the respiration of silkworms when bred on mulberry leaves to which cane sugar was added in different proportions.

As the results of the experiment we found that the activity of respiration is increased when bred on mulberry leaves to which cane sugar was added suitably and decreased when excess sugar was added.

Part VIII. The Change in the metabolism when bred on mulberry leaves to which cane sugar was added suitably and the consideration on the fate of digested sugar.

(1) Total digestion, digestion per 10 hours of the protein and carbohydrate are increased.

(2) The protein, glycogen and fat accumulated in the body per 10 hours are increased.

(3) The consumption of protein is decreased, but carbohydrate consumption is increased.

(4) 8.51% of digested sugar is changed to glycogen, 40.15% to fat and 51.68% is decomposed to CO_2 and H_2O.

Part IX. The digestion of the carbohydrates contained in leaves of several kinds of mulberry trees.

The digestion and digesitibility of carbohydrate, cane sugar and reducing in leaves are positively correlated to the sugar content.

Bergenin from Mallotus Japonicus, J. Mueller.

(pp. 394~396)

By K. HOMMA.

(Agricultural Chemical Society of Japan; Received Mar. 13, 1939.)

The author has isolated from *Mallotus Japonicus*, J. Mueller, a tree native to Japan and belonging to Euphorbiaceae, a crystalline substance, which shows every property of bergenin, desciibed by A. E. Tschitschibabin and his co-workers.

Futher identification was given by the elemental analysis (C 51.22 proct.; H 5.14 proct.;), methoxyl-number (7.90 proct.;), acetyl-number (38.88 proct.;) in the acetylated substance and lastly by the unaltered melting point with the original specimen of bergenin, which was sent to Assistant Prof. S. Hattori in the Phytochemical Laboratory of the Tokyo Imp. Univ. by Prof. Tschitschibabin himself some years ago.

Über die Bestimmung des Glutathions in Geweben.

(ss. 397~402)

Von Akiji FUJITA.

(Aus dem Biochemischen Laboratorium des Kitasato-Instituts.
Eingegangen, am 6, Apr., 1939.)

Biochemical Studies on Bakanae Fungus of the Rice. Part III.

Studies on Physiological Action of Gibberellin on the Plant.

(pp. 403~413)

By Teijiro YABUTA and Takeshi HAYASHI.

(Imperial Agricultural Station; Received Apr. 8, 1939.)

The physiological action of gibberellin, the active principle which makes the rice seedlings abnormally elongate, upon young shoots of several kinds of plants was tested by means of three different methods as below:—

(A) **Water culture experiment.**

Young shoots, 5 cm in length, were subjected to the flask culture with the solution containing 0.14～3.5 mg of gibberellin in 100 ccm water culture solution for about 10 days. The plants used were barley, buckwheat, ryokuto (Phaseolus radiatus, var. typicus, Prain), rape seed, hechima (Luffa cylindrica Roem), tomato, cucumber, cucurbit and morning glory.

(B) Injection experiment.

After Zimmerman and his coworker's experiment,(1) the aqueous solution of gibberellin was injected to the shoots by means of a glass tube drawn to a capillary at one end. The plants used were cucumber, cucurbit, morning glory, soy bean and azuki (Phaseolus anglaris, Wight).

(C) Lanolin application experiment.

After Zimmerman and his coworker's experiment, gibberellin was mixed with lanolin and applied to the basal portion of the shoots of cucumber with a glass rod.

In the above 3 experiments, the plants treated with gibberellin showed abnormal elongation similar to rice seedlings.

Lamna paucicostata, Hegelm, a floating weed found in the rice field, was cultured in solutions containing 0.14～3.5 mg gibberellin in 100 ccm solution with precautions as to constancy of pH and temperature, avoidance of contamination. After 18 days' culture the plant number, frond number and dry weight were estimated. The reproduction rate and dry weight of the plant cultured in solutions containing gibberellin were much greater than those in control solution.

(1) P. W. Zimmerman, F. Wilcoxon: Contr. B. Thompson Inst. Plant Res., **7**, 209～229, (1935).

On the Koji-Amylase.

Part XIII.—Effect of the Variety of Aspergillus Oryzae employed in Saké Manufacture upon the Formation of Amylases, Maltase and Protease in Saké-koji.

(pp. 414～418)

By Yuzo Tokuoka.

(Tsunekichi Okura Brewery. Received Jan. 31, 1939.)

It was clearly pointed out that the cultural characteristics of Asp. oryzae and the external appearance of saké-koji (Asp. oryzae grown on steamed rice) were quite different according to the various straines of Asp. oryzae.

However, no remarkable change in the formation of enzyme including α- and β-amylases, maltase or protease in saké-koji was found, although the formation of α amylase was suggested as being inversely proportional to the ratio of between the amounts of β- and α-amylases and of between maltase and α-amylase.

The relative activity of α- and β-amylases or maltase in various sakè-koji at 40° in pH 5.0 was found to be 100 : 19.5～11.2 (av. 15.4) : 9.3～6.2 (av. 7.5).

Therefore the relative activity of the above enzymes was again found to be 100 : < 2 : < 1 with such other koji-preparations as soy-bean, rice and wheat brans, since the relative activity of β-amylase and maltase was found to be about 10 times higher with saké-koji as mentioned in the previous paper [see This Journal. **14**, 839 (1938)].

Vol. 15 May 1939 No. 5

Bulletin of the Agricultural Chemical Society of Japan.

TRANSACTIONS

Studies on Vitamin B_2 Complex.

IV—Effect of Carbohydrate on Vitamin B_2 Deficiencies.

By Ume TANGE.

From The Institute of Physical and Chemical Research.

(Received October 29, 1938.)

During the experiments on vitamin B_2 deficiencies, it was found that there was a marked difference in the nutrition of young rats receiving vitamin B_2 complex deficient diets containing corn-starch, as compared to other rats receiving comparable diets containing sucrose as the source of carbohydrate, which was probably due to the minute quantity of some factor (or factors) of vitamin B_2 held by the starch particles.

Morgan, Cook and Davison,(1) Mitchell and Dodge(2) reported that high lactose ration had cataractogenic effect on rats, whereas Day, Langston and O'Brien(3) who used the more usually employed corn-starch as the basal carbohydrate, showed that flavin deficiency in the ration produced cataract in rats. There are several reports that while cataracts were more or less regularly produced on lactose-containing diet, cataracts resulting from vitamin G deficiency were not observed. This led to the use of sucrose as well as of lactose and corn-starch with varying single and multiple B_2 deficiencies.

This paper reports the effects of the three basal rations with sucrose, corn-starch or lactose as carbohydrate constituent on the vitamin B_2 deficiency disease occurring in the rat.

EXPERIMENTAL.

The basal rations had the following composition: Purified fish protein 18 parts; carbohydrate (sucrose, corn-starch or lactose) 68 parts; crisco 9 parts; McCollum's salt mixture 4 parts. In some of the lactose diets a lower proportion of fish protein was used, 10 parts, but in total amount 20 parts by adding 10 parts of dried egg-white powder. The crisco content in the sucrose diets was reduced from 9 to 3 parts in the later experiments and 2 parts of powdered agar-agar were added. The lactose was purified by repeating extraction with hot 94 per cent alcohol. The corn-starch was exposed to strong day-light in thin layer for 10 days before being incorporated in the diet.

Rats weighing 55 to 65 g were placed on the basal diets, provided daily with one drop of cod liver oil and 10~20 γ of vitamin B_1 according to body weight, care being taken in the group distribution as described in the preceding paper.[4]

Flavin, vitamin B_6, and filtrate factor, singly or in combination of the two or three, were used as the vitamin B_2 factors.

Flavin was given as egg-white powder at the level of 6 per cent, except 10 per cent in the lactose ration.

Vitamin B_6 and filtrate factor concentrates were fed in daily amounts per rat equivalent to 1 g dried yeast.

Preparation of Supplementary Materials.

Vitamin B_6 concentrate was prepared as follows: 500 g of dried brewer's yeast were extracted twice with 1600 cc of cold 70 per cent alcohol and three times with 1500 cc of boiling 80~85 per cent alcohol. The combined extracts were concentrated in vacuo to 500 cc, and the solid and the fats removed by filtering and ether extraction. The solution was then adjusted to pH 2.0 with H_2SO_4 and treated with 80 g of acid clay to remove vitamin B_1 and flavin. The first adsorbate was rejected, and the filtrate was treated twice with 85 g portions of acid clay. The second and third adsorbates, after being washed with water and alcohol, were eluted with 0.2 *N* $Ba(OH)_2$. The resultant solution was freed from barium by addition of H_2SO_4 and concentrated under reduced pressure (1 cc = 2 g yeast, dry wt.). The eluate was used as vitamin B_6.

Filtrate factor: The final filtrate, from the acid earth adsorbate of yeast extract mentioned above, was treated with $Ba(OH)_2$ to remove H_2SO_4 and concentrated in vacuo to 250 cc (1 cc = 2 g yeast, dry wt.).

The Effect of Lactose.

The outstanding feature in the groups on the lactose diet was the complete absence of dermatitis, frequent appearance of cataract, and slightly subnormal growth, but good nutritional condition as the control rats, even with the entire B_2 deficient diet. Young rats when first put on this ration often had a diarrhea which subsided later, although the stool frequently continued to be softer than that of rats on other carbohydrate diet.

By giving vitamin B_6 (it being understood that B_1 is present in all cases) no growth improvement was brought out. The addition of dried egg-white powder as flavin at the level of 10% to the low protein ration composed of 10 parts of fish protein, resulted in some increase of growth rate. When filtrate factor with the other two factors was given, the growth was, however, definitely stimulated and the appearance of cataract was somewhat delayed. Even with the administration of 4% dried brewer's yeast, at the beginning of the experiment, the development of cataract could not be prevented.

It is of interest that vitamin B_6 and flavin along with the 68% lactose diet

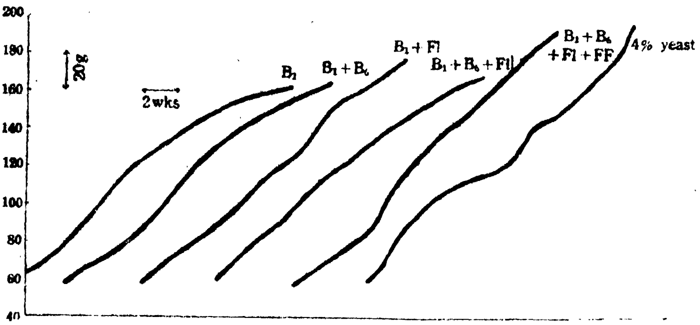


Chart 1. Average growth curves of rats on lactose diet with one, two, three or four of the vitamins: B_1, flavin (Fl), filtrate factor (F,F) vitamin B_6(B_6).

brought about little improvement on the growth. This phenomenon may perhaps be attributed to the fact that lactose favours the production of both flavin and vitamin B_6 in the intestine probably by microorganisms as indicated in the experiment of Morgan and his associates(5) with the alcohol extract of ceca content of the lactose-fed rats on the rats depleted on the corn-starch diet.

The Effect of Corn-starch.

The growth of the rats on corn-starch diet without supplements was very slow, sometimes dermatitis developed but none of the animals has shown any cataract. By the addition of B_6 or flavin singly or in combination, the growth was not greatly stimulated, in spite of the fact that administration of flavin prevented alopecia, a denuded condition, called a "skin affection" by Chick, Copping, and Edgar.(6) However, the addition of filtrate factor after growth had ceased on flavin gave a marked growth response and improvement of the symptoms; filtrate factor alone produced little improvement. This result confirmed also the previous finding.(3) The phenomenal growth response resulting from the addition of flavin and filtrate factor to the corn-starch diet and the relative ineffectiveness of the addition of vitamin B_6 to flavin gave us the supposition that corn-starch carried appreciable amounts of vitamin B_6. Indeed recent reports of Dann(7) and Copping(8)

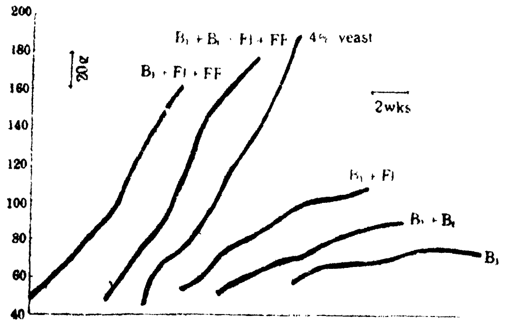


Chart II. Average growth curves of rats on cornstarch diet with one, two, three, or four of the vitamins: B_1, flavin (Fl), filtrate factor (FF), vitamin B_6 (B_6).

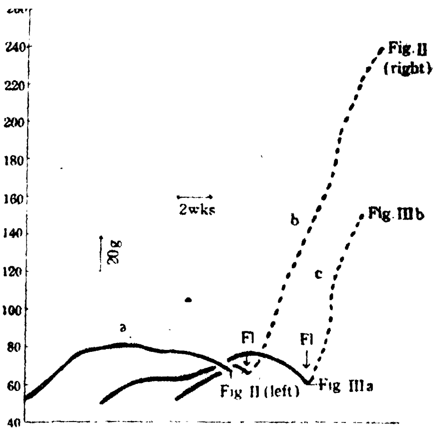


Chart III. Growth curves of rats on cornstarch diet containing 5% cane molasses, as source of filtrate factor and B_6. Fl (flavin).

showed that corn-starch is a good source of vitamin B_6 and that one-third to one-quater of the vitamin B_6 present is extracted by cold 80% alcohol.

In an earlier experiment, a group of rats was fed on the corn-starch ration containing 5% cane molasses which was previously tested to be absent of flavin, but to contain vitamin B_6 and filtrate factor. The rats ceased to grow after about 4 weeks and maintained themselves at 70 g for a prolonged period. The animals showed very characteristic alopecia and severe sore eyes, but had no cataract, When flavin (at the level of 6% dried egg-white powder) was given, the animals immediately resumed weight, and the symptoms disappeared as seen in Chart III and the photographs (Figs. 2 and 3). The rat receiving flavin from the earlier stage of the experiment was also included for comparison. This was entirely different from the results of Day, Darby, and Langston(9) who have consistently produced cataract on flavin deficient diet. We must realize, therefore, that cataract in rats resulting from flavin deficiency is apparently different in etiology from the cataract which results from lactose feeding, as pointed out by Day, Darby, and Cosgrove.(10)

The Effect of Sucrose.

The rats fed on the sucrose ration without supplement (it being understood that vitamin B_1 is present in all cases) showed little or no gain, and survived for only several weeks. The addition of flavin produced growth stimulation for some interval, but developed the acrodynia-like dermatitis due to the deficiency of vitamin B_6. If this condition continued the rats would lose their weight and die. The survival period of the animals, however, was greatly influenced by the amounts of the fat contained in the basal ration, in other words, the fat exerts a remarkable sparing action on vitamin B_6 (the relationship between vitamin B_6 and fats will soon be reported).

Obviously the ration must be supplemented with one or more of the factors of vitamin B_2 complex. When vitamin B and flavin were given together, the rats failed to attain normal growth, their weight being subnormal. When both flavin and filtrate factor were given, the animals continued to grow, but often developed the acrodynia-like dermatitis. When all three factors were given, normal growth

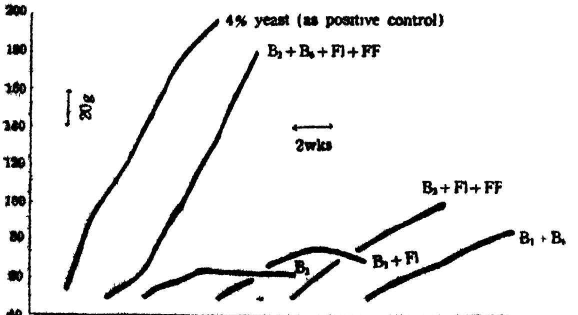


Chart IV. Average growth curves of rats on sucrose diet with one, two, three, or four of the vitamins. B_1, flavin (Fl), filtrate factor (Fl), vitamin B_6 (B_6).

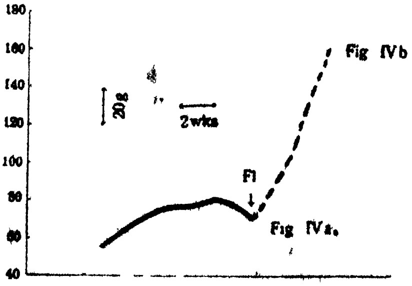


Chart V. Growth curves of rats on sucrose diet containing 5% cane molasses, as source of filtrate factor and B_6. Fl (flavin).

was produced nearly as good as that on the basal ration with 4% yeast.

In an experiment, a group of rats was fed on the sucrose diet containing 5% cane molasses. The rats were allowed a little growth, no cataract developed, but severe alopecia and sore eyes occurred. These symptoms were immediately cured by administration of flavin. It is probable that the cane molasses was lacking chiefly in flavin. This is demonstrated with the growth curve as well as the photographs below.

The choice of carbohydrate, therefore, as a part of the basal ration would seem to be very important in the study of the vitamin B_2 deficiency. There is certain evidence that the depletion period was shorter and deficiency symptoms more severe with sucrose instead of corn-starch: moreover it was shown that the different influences of sucrose and dextrine on growth and dermatitis appeared in the rat.(11)(12)

SUMMARY AND CONCLUSIONS.

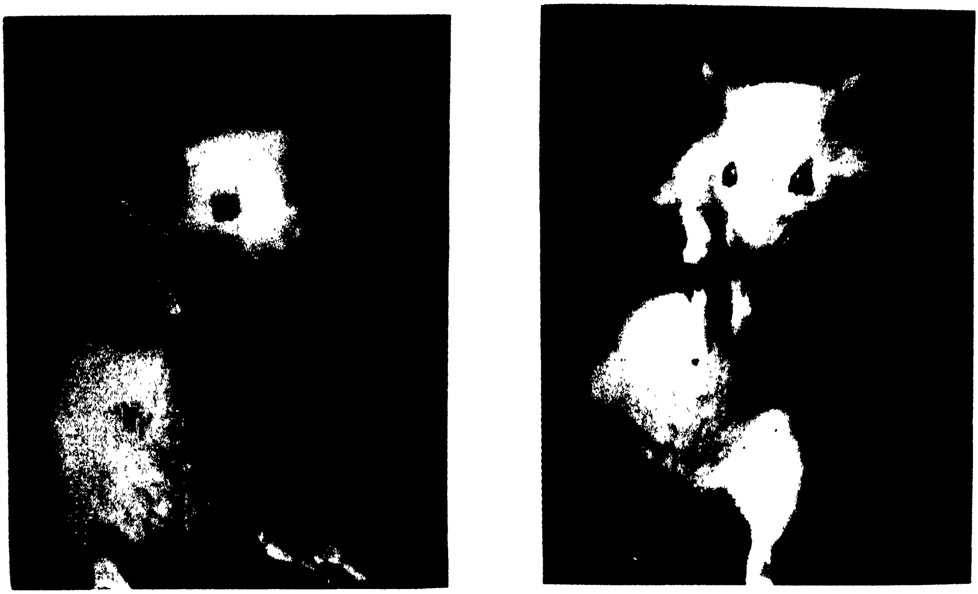
1. Lactose had an unique effect on the nutrition of rats. None of the lactose-fed rats developed dermatitis, but cataract. The animals continued to grow even on the entire B_2 deficiency. Vitamin B_6, flavin or filtrate factor separately administered did not give any appreciable improvement on either growth or cataract. When all three factors, B_6, flavin, and filtrate factor, were supplied, a definite effect on growth was produced and slower development of cataract was brought about.

2. The rats fed on the corn-starch diet failed to grow and developed alopecia unless flavin and filtrate factor were supplied. Vitamin B_6 had little effect on improvement of the growth rate. This factor appeared to be present in the starch. When fed on the corn-starch diet containing 5% cane molasses, the rats maintained themselves at 70 g for a prolonged period and developed severe alopecia and sore eyes, but no cataract.

3. When sucrose was used as the basal carbohydrate, earlier and severer development of dermatitis and growth failure occurred unless vitamin B_6, flavin and filtrate factor were supplied. No cataract appeared on this diet. Apparently sucrose is completely free from all three factors and is therefore the carbohydrate of choice for the study of the vitamin B_2 deficiency.

I wish to thank Dr. U. Suzuki for his advice and encouragement during the progress of this work. I am also grateful to Miss T. Kaneko for her willing help in feeding the animals and preparing the diets.

(1) A. F. Morgan, B. B. Cook and H. G. Davison: J. Nutrition, **12**, 37 (1934)
(2) H. S. Mitchell and W. M. Dodge: J. Biol. Chem., **105**, LXI (1934)
(3) P. L. Day, W. C. Langston and C. S. O'Brien: Am. J. Ophth., **14**, 1005 (1931)
(4) Ume Tange: Sc. Pap. I. P. C. R., **35**, 47 (1938)
(5) A. F. Morgan, B. B. Cook and H. C. Davison: J. Nutrition, **15**, 27 (1938)
(6) H. Chick, A. M. Copping and C. E. Edgar: Biochem. J., **29**, 722 (1935)
(7) W. G. Dann: J. Nutrition, **11**, 451 (1936)
(8) A. M. Copping: Biochem. J., **30**, 849 (1936)
(9) P. L. Day, W. J. Darby and W. C. Langston: J. Nutrition, **14**, 389 (19
(10) P. L. Day, W. J. Darby and K. W. Cosgrove: J. Nutrition, **15**, 83 (1938
(11) N. Halliday and H. M, Evans: J. Biol. Chem., **118**, 2555 (1937)
(12) Ume Tange: Rikwagaku-kenkyu-jo Iho, **16**, 1058 (1937)
(13) Ume Tange: Sc. pap. I. P. C. R., **35**, 56 (1938)



A. Normal

B. Rat with cataract resulting from the 68% lactose.

Fig. 1.

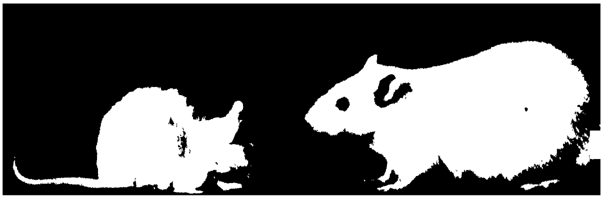
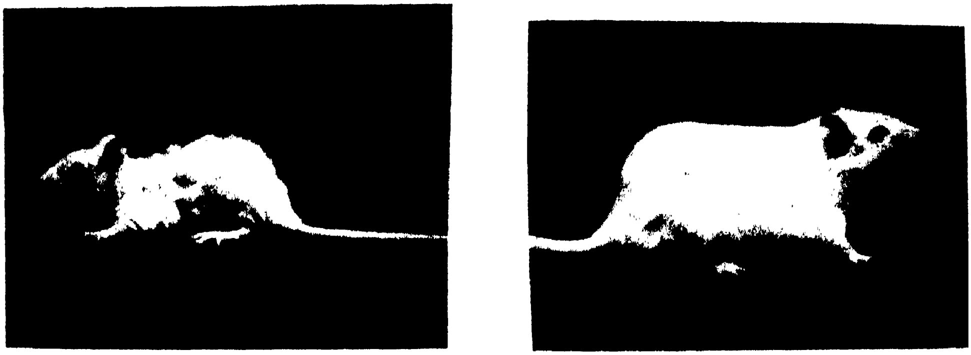


Fig. 2. Left—Rat fed on the corn-starch diet in flavin deficiency: alopecia and sore eyes, but no cataract.

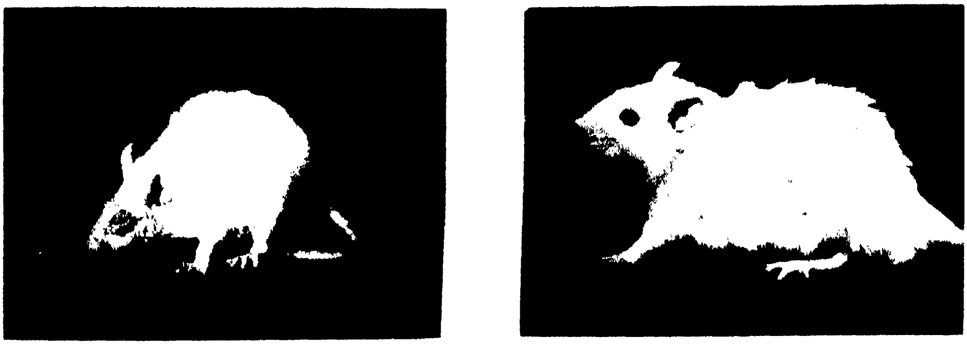
Right—Rat fed on the corn-starch diet with flavin: normal growth. These rats were photographed on the seventy-seventh day of experiment, at which time they weighed 68 g and 250 g, respectively



Rat—a, Showing alopecia and sore eyes resulting from flavin deficiency, but no cataract (60 g).

Rat—b, Showing recovery of the rat (a) by administration of flavin (151 g).

Fig. 3.



Rat—a, Showing alopecia and sore eyes resulting from flavin deficiency (70 g).

Rat—b, Showing recovery of the rat (a by administration of flavin (162 g).

Fig. 4

ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

On the Stimulant for Cane Sugar Formation in Plants. (II)

(pp. 419～421)

By Tetutaro TADOKORO and Naomoto TAKASUGI.

(Biochem. Lab., Faculty of Sci., Hokkaido. Imp. Univ.; Received Apr. 7, 1939.)

Über die Verschiedene Eigenschaften von Aspergillusarten. (VI)

(ss. 422～428)

Von T. TAKEDA und O. TAKEUTI.

(The Department of Industry, Government Research Institute, Taiwan, Nippon; Received Apr. 19, 1939.)

Researches on Bamboos as a Raw Material for Pulp in Taiwan.

(pp. 429～434)

By Minoru TUTIYA and Setuo HUKUHARA.

(Industrial Research Institute of Taityu; Received Apr. 9, 1939.)

The Influence of Monochromatic Lights on the Action of Enzymes.

(pp. 435～444)

By Reitaro MURAKAMI.

(Agricultural College, Utunomiya; Received Jan. 31, 1939.)

[Report XVII.] Especially on the Influence of Infra-red Rays on the Action of Enzymes. (Part 1.) Yeast Saccharase.

To investigate the influence of infra-red rays on the action of the saccharase in the yeast, the author has employed "Vim Ray" red lamp. The preparation of the enzyme, the addition of the enzyme solution into the substrate, the determination of the end product by the action of the enzyme and other treatments were carried out as in the author's previous papers [Report XII], but the filters at the front of the boxes containing test tubes lighted by "Vim Ray" red lamp were different.

The transmission wave lengths of white, infra-red pass and black screens used as the light filter were > 5700 1.Å., > 8000 1.Å. and none, respectively.

In this experiment, the action of the yeast saccharase was found to be promoted by infra-red rays. The enzyme under the rays containing both visible and infra-red was more active.

[Report XVIII.] Especially on the Influence of Infra-red Rays on the Action of Enzymes.

(Part 2.) Yeast Amylase.

As a light source "Vim Ray" red lamp was employed. The light filters were the same as in the author's previous paper [Report XVII]. The preparation of the enzyme, the addition of the enzyme solution into the substrate, the determination of the end product by the action of the enzyme and other treatments were the same as in the author's previous paper [Report XIII].

In this experiment, the action of the yeast amylase was found to be promoted by infra-red rays, but the enzyme was more active under the rays containing both visible and infra-red.

[Report XIX.] Especially on the Influence of Infra-red Rays on the Action of Enzymes.

(Part 3.) Yeast Proteinase.

As a light source "Vim Ray" red lamp was employed. The light filters were the same as in the author's previous paper [Report XVII]. The preparation of the enzyme, the addition of the enzyme solution into the substrate, the determination of the end product by the action of the enzyme and other treatments were carried out as in the author's previous paper [Report XIV].

In this experiment, the action of the yeast proteinase was found to be promoted by infra-red rays, but the enzyme under the rays containing both visible and infra-red was more active.

[Report XX.] Especially on the Influence of Infra-red Rays on the Action of Enzymes.

(Part 4.) Yeast Lipase.

"Vim Ray" red lamp was employed as a light source. The light filters were the same as in the author's previous paper [Report XVII]. The preparation of the enzyme, the addition of the enzyme solution into the substrate, the determination of the end product by the action of the enzyme and other treatments were the same as in the author's previous paper [Repert XV].

In this experiment, the action of the yeast lipase was found to be promoted by infra-red rays, but not so much as by the rays containing both visible and infra-red.

[Report XXI.] Especially on the Influence of Infra-red Rays on the Action of Enzymes.

(Part 5.) Yeast Catalase.

As a light source "Vim Ray" red lamp was employed. The light filters were the same as in the author's previous paper [Report XVII], and the preparation of the enzyme, the addition of the enzyme solution into the substrate, the determination of the end product by the action of the enzyme and other treatments were carried out as in another previous paper. [Report XVI].

In this experiment, the action of the yeast catalase was found to be promoted by infra-red rays, though not so much by the rays containing both visible and infra-red.

Studies on the Utilization and Digestion by Silkworms of the Carbohydrates Contained in Mulberry Leaves.

(pp. 445~458)

By K. Kato.

(The Sericultural Experiment Institute of Gifu Prefecture; Received Mar. 8, 1939)

Part X. Research on the content of carbohydrates in mulberry leaves.

The content of carbohydrates in mulberry leaves varied with kinds of mulberry trees, degree of growth and quantity of manure, etc. In this research, the largest value of total carbohydrate content is 10.60%, smallest is 1.62% and the arithmetical mean value is 4.72%.

Part XI. The Relation of the sericultural harvest and the carbohydrate content of mulberry leaves.

The sericultural harvest was best when bred on mulberry leaves which have 3~4% sugar content at first instar, and 4~5% sugar content at second instar.

Part XII. Devise of the apparatus for determination of carbohydrate content in leaves.

As the results of various experiments, I devised an apparatus for determination of carbohydrate content in leaves. This apparatus is accurate and convenient for use at any place not equipped as a chemical laboratory.

Studies on Alkaline Earth. I.

(pp. 459～472)

By Kinjiro KAWASE, Hogai KA and Kozo KAWAKAMI.

(The Institute of Scientific Research, Manchoukuo; Received Apr. 10, 1939.)

In the alkaline district of North Manchuria, a kind of edible salt, "earth salt" or "tou-en" is manufactured from earth.

The earth, raw material of the salt, is extracted with water, filtered and evaporated, and by these primitive processes the earth salt is manufactured.

After a study of the physical and chemical properties and field observations on various earth salts, the following conclusions were obtained.

(1) The original earth which is used by the natives for the preparation of the earth salt contains about 24.895% of water soluble matters.

(2) The cation of the chemical constituents of the water soluble matters contains mainly $Na^{\cdot}$ (99%～62%), while the hygroscopic alkali spot contains chiefly $Ca^{\cdot\cdot}$, $Mg^{\cdot\cdot}$ (35%).

The amount of anions (Cl', SO_4'', CO_3'', HCO_3') varies in accordance with the outside conditions.

The surface deposits visible to the naked eye contain more SO_4'' than Cl', while the soil 1 cm below contains more Cl' than SO_4''.

The deposit near the lake and the reddish-brown deposit which is rich in CO_3'', HCO_3', on the contrary, contain more Cl' than SO_4''. The earth collected by natives for the production of earth salt contains extraordinarily more Cl' than SO_4''. With CO_3'', HCO_3' no such particular properties can be seen as in the case of Cl' and SO_4''. Moreover they can be distinguished from the sea-salt by the fact that they all contain considerable quantities of NO_3', NO_2'.

The Utilization of the By-products of Soy-beans. (Part V)

(pp. 473～477)

By Yosaburo IWASA.

(Dept. of Food Chemisty, Osaka Municipal Hyg. Lab.; Received Apr. 15, 1939.)

Studies on Fumigants. Part I. The Reaction Produets of Trichlorethylene and Nitric Acid. No. 1.

(pp. 478～482)

By M. KOHNO and T. SAKAI.

(Sankyo Chemical Laboratory, Osaka; Received Apr. 28, 1939.)

Studies on the Etherification of Ethyl Alcohol.

Part I. Preliminary Experiments.
Part II. Determination of Ether by Distillation

Zuckerrohrwachs. III. Mitteilung.

Neue Bestandteile der unverseifbaren Substanz des Zuckerrohrwachses.

(ss. 526∼530)

Von T. Mitui.

(Aus dem Agrikulturchem. Laboratorium der Kaiserl. Universität, Kyoto;
Eingegangen am 10 Mai 1939)

In meiner I. Mitteilung berichtete ich, daß es mir gelang, aus der unverseifbaren Substanz des Zuckerrohrwachses Sitosterin und Stigmasterin zu gewinnen. Meine weiteren Untersuchungen nun liessen mich zwei neue Bestandteile in der unverseifbaren Substanz des Zuckerrohrwachses finden, welche folgenden Charakter haben:

	Schmelzpunkt	Formel	Doppelbindung	Natur der Sauerstoffe
I	206°	$C_{29}H_{52}O_2$	0	2 —OH
II	106°	$C_{29}H_{48}O$	1	1 —CO—

Ihren Eigenschaften nach kann man die Stoffe chemisch als dem Sterin verwandte Stoffe betrachten. Daher mochte ich nach seinem Vorkommen im Zuckerrohr (Saccharum officinarum) den I. Stoff *"α-Saccharostandiol"* und den II. Stoff *"β-Saccharostenon"* nennen.

Die Schmelzpunkte ihrer Derivate sind:

α Saccharostandiol-dibenzoat	160°
〃 -di-m-dinitrobenzoat	171°
β-Saccharostenon-oxim	163—165°
〃 -2, 4-dinitrophenylhydrazon	206°
〃 -tribromid	160 5°

Studies on the Preparation of Unsaturated Higher Fatty Alcohol by Zinc Catalyst.

(pp. 531∼541)

By Yuichi Shinozaki and Shizuo Sumi.

(Dept. of Organic Chem., the Central Laboratory, South Manchuria Railway Co., Dairen,
Received May 11, 1939)

Part I. On the Formation of Unsaturated Higher Fatty Alcohol from Perilla Oil.

Part II. The Influence of the Addition of Lower Aliphatic Alcohol upon the Catalyst.

Chemical Researches on the Pulp Woods of Manchoukuo. Part V.

Chemical Analysis and Cooking Experiment of "Dahurica-Karamatsu".

(pp. 542∼546)

By M. Shikata, M. Yamane, and H. Kin.

(Agricultural Chemical Institute, Kyoto Imperial University,
Received May 27, 1939.)

In this paper, the researches on the chemical components, fibre lengths, and cooking experiments of Dahurica-Karamatsu (Howan-Howasun, *Larix dahurica* Turcz) are given.

1. The fibre length.

The distribution of fibre length is as shown in Table 1.

Table 1. The Distributions of Fibre-length.

Length (mm)	Dahurica-Karamatsu (%)	Length (mm)	Dahurica-Karamatsu (%)
0.5~1.0	1.4	3.5~4.0	14.2
1.0~1.5	4.8	4.0~4.5	13.2
1.5~2.0	4.6	4.5~5.0	5.0
2.0~2.5	13.2	5.0~5.5	0.4
2.5~3.0	21.2	5.5~6.0	0.2
3.0~3.5	20.8	6.0~6.5	0.4

The data show that the fibre length of Dahurica-Karamatsu is nearly equal in length to those of soft woods.

2. Chemical components.

The results of the analysis are given in Table 2.

Table 2. Chemical Components. (abs.)

The place of production	Hing-an, Peh-Sheng	Hing-an, Tung-Sheng	The place of production	Hing-an, Peh-Sheng	Hing-an, Tung-Sheng
Alcohol-benzene soluble	3.70	2.05	β-Cellulose	3.68	11.30
Cold water soluble	20.22	7.45	γ-Cellulose	10.12	6.04
Hot water soluble	21.31	8.94	In total cellulose α-Cellulose	72.90	69.26
1%NaOH soluble	29.61	17.82	In total cellulose β-Cellulose	7.23	20.04
Galactan	8.93	2.39	In total cellulose γ-Cellulose	19.87	10.70
Mannan	5.77	2.34	Nitrogen	0.22	0.14
Pentosan	11.78	6.66	Crude Protein	1.39	0.88
Hemicellulose	26.48	11.39	Ash	0.35	0.21
Lignin	25.96	28.96	CH_3O	4.58	4.50
Total cellulose	50.92	56.40	CH_3O/Lignin × 100	17.64	15.54
α-Cellulose	37.12	39.06			

3. Cooking experiments.

The cooking experiments with Ca-sulphite process were carried out under the conditions given in Table 3.,

The compositions of cooking liquor was as follows;

Total SO_2 . . . . 7%; CaO 1%;

Ratio of liquor and air dry sample is 5 to 1 in weight.

Table 3. The Conditions of Cooking Experiments.

The place of production		Hing-an, Peh-Sheng			Hing an, Tung-Sheng		
No		D 97	D 98	D 3	D 97	D 98	D 5
Penetration	Temp. °C	110~120	105~120	110~120	110~120	105~120	110~120
	Time hr	2	1 1/2	3	2	1 1/2	3
Main Cooking	Temp °C	135	135	135	135	135	135
	Time hr	6	5	5	6	5	5
	Press kg/cm^2	6	6	6	6	6	6

4. Properties of unbleached pulps are shown in Table 4.

Table 4. Properties of Unbleached Pulps. (abs.)

The place of production	Hing-an, Peh-Sheng			Hing-an, Tung-Sheng		
No	D 97	D 98	D 3	D 97	D 98	D 5
Yield	38 80	43 66	40 40	42 10	44 00	41 59
Screenings			2 00			2 33
Total cellulose	95 47	96 70	95 76	96 85	96 09	95 19
α-Cellulose	84 44	87 03	84 62	86 21	84 63	83 02
β Cellulose	8 94	4 17	6 44	4 43	5 80	5 21
γ-Cellulose	2 09	5 50	4 70	6 21	5 66	6 96
Ash	0 37	0 44	0 66	0 21	0 46	0 72
Roe's number	4 83	3 04	5 61	1 45	4 45	5 98

5. Bleached pulp.

The unbleached pulps were bleached by the two stages method. After applying chlorine gas, they were washed with diluted alkali and water, and steeped in bleaching powder solution.

The properties of bleached pulps are shown in Table 5.

Table 5. Properties of Bleached Pulps. (abs.)

The place of production	Hing-an Peh-Sheng			Hing-an, Tung-Sheng		
No	D 97	D 98	D 3	D 97	D 98	D 5
Yield	36 62	41 56	35 87	38 65	41 34	36 92
Yield to 1 m^3 wood kg	207 4	235 4	203 17	208 2	211 9	189 22
α-Cellulose	91 22	91 06	92 08	90 84	89.53	90.85
β-Cellulose	5 13	3 37	3 10	3 32	4 87	3 18
γ-Cellulose	3 65	5 57	4 82	5 84	5 60	5 98
Ash	0 20	0 15	0 24	0 11	0 18	0 24
Pentosan	4 08	4 39	0 65	4 23	4 40	1 77
Alcohol benzene soluble			0 24			0 15
Copper number	0 50	0 61	1 23	0 84	0 92	1 17
Viscosity (centipoises)	15 0	38 0	27 7	28.1	23.8	45 0

We may conclude from Table 5. that the pulp from Dahurica-Karamatsu is not inferior to first class commercial rayon pulps.

Experimentelle Untersuchungen über die Wirkung von Radium- und Röntgen-Strahlen auf die Gärungsmikroorganismen (II. Mitt.)

Über die Saurebildung der *Asp. niger* Radiumrassen.

(ss. 547～552)

Von R. NAKAZAWA und M. SIMO.

(Department of Industry, Government Research Institute, Taiwan, Japan;
Received May 15, 1939.)

A Simple Colorimetric Method to estimate Vitamin A.

(pp. 553～562)

By Dr. Kokichi OSHIMA and Shinichi ITAYA.

(From the Chemical Laboratory of the Hakodate College of Fisheries, Japan;
Received June 5, 1939.)

Instead of the Lovibond tintometer, a set of closed tubes which contain the following standard solutions is used:

Unit with Lovibond tintometer				Contents
Blue	10.0	Yellow	2.2	25{34% $CuSO_4 \cdot 5H_2O$}+4{11.3% $Co(NO_3)_2 \cdot 6H_2O$}
〃	9.0	〃	2.0	25{30% $CuSO_4 \cdot 5H_2O$}+4{10% $Co(NO_3)_2 \cdot 6H_2O$}
〃	8.0	〃	1.8	86 [25{30% $CuSO_4 \cdot 5H_2O$}+4{10% $Co(NO_3)_2 \cdot 6H_2O$}]+14 Aqua
〃	7.0	〃	1.5	72 [〃]+28 〃
〃	6.0	〃	1.3	58 [〃]+42 〃
〃	5.0	〃	1.15	44.6 [〃]+55.4 〃
〃	4.0	〃	1.0	32 [〃]+68 〃
〃	3.0	〃	0.74	21 [〃]+79 〃
〃	2.0	〃	0.5	12 [〃]+88 〃
〃	1.0	〃	0.4	5 [〃]+95 〃
〃	0.8	〃	0.32	4 [〃]+96 〃
〃	0.6	〃	0.24	3 [〃]+97 〃
〃	0.4	〃	0.16	2 [〃]+98 〃
〃	0.2	〃	0.08	1 [〃]+99 〃

In a test tube of the same size as the standard, 0.5 cc liver oil in chloroform is mixed with 4.5 cc of 20% antimony trichloride in chloroform. Compare the blue color of the mixture with the standard in a comparator box. This comparison should be completed within 30 seconds. The vitamin A value of any given liver oil is expressed as follows:

$$\text{C. L. O. unit} = \frac{\text{Obtained blue unit}}{\text{\% of liver oil (wt/vol)}} \times 2$$

$$\text{Carr Price unit} = \frac{\text{Obtained blue unit}}{\text{\% of liver oil (wt/vol)}} \times 2 \times 9.1$$

$$\text{International unit} = \frac{\text{Obtained blue unit}}{\text{\% of liver oil (wt/vol)}} \times 2 \times 300$$

If the color of the mixture shows some different tones from the standard, then use also the yellow or red standard tubes adopted in the authors' method of estimating the fatty oil color.

The entire apparatus can be carried about easily, and the colors of the standard solutions do not fade even under direct sunlight.

On the Manufacture of Alcohol from Jerusalem Artichoke. (Part X.)

(pp. 563~574)

By Toshinobu Asai and Susumu Kikuchi.

(Agricultural Chemical Laboratory, Morioka Agricultural College, Japan,
Received May 24, 1939)

The influence of the addition of nitrogenous substances to the alcoholic fermentation of the unsaccharified steamed mash was investigated. The mash was prepared so as to contain about 12~14% of total sugars.

It was found that peptone-nitrogen was most effective to promote the fermentation velocity and increased the alcohol yield to above 90% conversion, while extracted soy-bean and ammonium sulphate increased fermentation a little, though bran and rice bran rather inhibited fermentation. The optimum quantities of peptone-nitrogen to be added were found to be 0.1~0.4 grams per 1000 cc of the mash.

Potassiumbiphosphate gave rapid fermentation at initial period but the total alcohol yield was not increased.

The mixed culture of yeasts improved the fermentation, most especially with Schizosaccharomyces Pombe and alcohol yeast No. 35.

Lastly we investigated the fermentation of the aqueous extract produced by diffusion process. The dried artichoke chips were extracted in diffusion battery with water at approximately 80°C; a syrup was obtained, containing about 20% total sugar equivalent. Remaining carbohydrate after the extraction showed only 0.3% of the residual chips.

The extract without dilution was fermented by Shizosaccharomyces Pombe.

The fermentation result was most excellent showing the alcohol yield of 88.6% of the theoretical in the fermenting duration of 88 hours.

Statistic Studies of Soils (VII).

(pp. 575～588)

By Dr. MISU-Hideo.

(Agricultural Experiment Station, Government General of Tyôsen;
Received June 2, 1939.)

Über die chemischen Zusammensetzung und die Verhältnisse zwischen der Faktoren der Getreidefennichen Samen.

(ss. 589～601)

Von Tetsujiro OHARA.

(Tokyo Nogyo Kyoiku Senmon Gakko; Eingegangen am 27, Apr , 1939)

Über die Eigenschaften der Getreidefennichen Samen.

(ss. 602～608)

Von Tetsujiro OHARA.

(Tokyo Nogyo Kyoiku Senmon Gakko, Eingegangen am 27, Apr., 1939)

Studies on Acetone-Butylalcohol Fermentation.

(1). The bacteriological properties of Bacillus butanolo-acetoni Yukawa-Horie nov. sp. and the fermentation of cassava by this organism.

(pp. 609～623)

By Matao YUKAWA and Sigeyosi HORIE.

(Agricultural Chemical Laboratory, Kyusyu Imperial University, Fukuoka;
Received May 29, 1939.)

The authors isolated a new strain of "acetone bacillus", for which a group-name Clostridium acetobutylicum (Weizmann) was proposed by McCoy and others, from maize bought at a local market in 1930.

The bacteriological and biochemical properties of this new strain of "acetone bacillus" was studied comparing with the so-called Bac. granulobacter pectinovorum, which was isolated by C. Weizmann in 1915.

The culture of the Weizmann bacillus used in this research has been developed from stock-culture obtained through the kindness of Prof. Speakman of Canada.

These two organisms are very nearly related in many respects, but in some properties, i. e., the fermentability of rhamnose and trehalose; the form of sporangia; the fermentability at low temperature; the easiness with which a most vigorous seed culture may be prepared; etc., they do not coincide with each other and hence we named it Bacillus butanolo acetoni Yukawa-Horie nov. sp.

Lately Prof. Dr. I. Yamasaki and T. Karasima made clear the difference of ability of acetoin formation between Bac. granulobacter pectinovorum and our new strain.

The bacteriological properties of this organism are as follows·

1. Morphology.

Vegetative cells. Medium: 4% cassava mash containing 1% bran as N-source. Temperature 37°C. Age: 24 hrs. Form: Long rods. Arrangement: Single but occasional pairs or chains (3 cells). Limits of size: 3∼8 μ × 0.8∼1 μ. Size of majority: 4∼6 μ × 0.8 μ. Gram stain · Positive. Lugol's iodine: Negative. Motility: Motile.

Sporangia. Medium and temperature: The same as above. Age: 48 hrs. Form: Drum sticks. Arrangement: Single but occasional pairs. Limits of size: 6∼12 μ × 1.5∼2.5 μ. Majority of size: 6∼8 μ × 2 μ. Gram stain: Positive. Lugol's iodine. Positive. Motility: Non-motile.

Spores. Location. At one end of sporangium. Form: Elliptical form. Size · 2∼2.5 μ × 1.5 μ.

2. Cultural features.

Plate culture (aerobically and anaerobically on wort gelatine, wort agar, koji agar and maize agar): No growth.

Slant culture. Anaerobically on wort agar: Occasional growth. Koji agar: No growth. Potato: Abundant growth and gas formation in both aerobic and anaerobic tubes. Slimy, cream colored. Strong butyl odor. Sporangia are formed after 24 hrs.

Stab culture. Wort agar and koji agar: Abundant growth without exception. Gas splits the medium.

Milk. Rapid coagulation. Butyl odor.

Gelatine. Liquefied.

3. Physiology.

Temperature relation. Spores can be boiled (in water bath) 20 mins. Opt. temp. for fermentation is 37°C (±2°). 5.4% acetone on cassava was produced in 159 hrs. at 23°C. At 45°C., no fermentation.

Air relation. Vigorous growth in both aerobic and anaerobic tubes.

Fermentation of various carbon sources. The fermentation tests were carried out in yeast water and Speakman's semi-synthetic medium. The following are fermented: xylose, arabinose, glucose, fructose, galactose, mannose, sucrose, maltose, lactose, raffinose, melizitose, α-methyl-glucoside, glycogen, inulin, dextrin, starch, pectin, mannite, rhamnose(?), trehalose(?), esculin(?). The following are not fermented: melibiose, glycerin, sorbit, Ca-lactate.

Fermentation products from 100 kg. cassave. About 7 kg. acetone, 14 kg. butylalcohol and 2 kg. ethylalcohol (within 72 hrs.).

Raw-materials in industrial fermentations: Cassava, maize, rice, kaoliang, potato, sweet potato, waste products from starch manufacture and others. Molasses can also be fermented but very poorly.

When such a material as cassava, which contains very little protein, is used, one of the following is added as N-source: Gluten meal, bran, chrysalis cake, soy bean cake, etc. In spite of the good yield of solvents from rice, rice bran itself is unsuitable for N-source.

The maximum limit of mash concentration in industrial practice lies between 6 to 8% (cassava) and 0.5% or less of gluten meal.

Bac. butanolo-acetoni needs not to be started from sand culture for each fermentation, that is, practically the same yield of solvents can be obtained by inoculating a seed culture of 4th to 25th generation, which is heat-shocked at each subculture, or of 4th to 10th generation, which is transferred without heat-shock. In the early generation, the seed culture, transferred without heat-shock, gives a greater yield of solvents than with it.

From many points of view, it is advisable to lower the viscosity of starchy mashes, especially in large scale fermentation, and the addition of a small amount of acid (sulphuric, acetic, etc.,) —0.02% on mash—to mash before cooking is the most simple and effective means for this purpose.

Employing this new strain, an acetone and butylalcohol producing factory was established in Japan for the first time in 1931.

Studies on the Cell-Wall Constituents of Soy-Bean. (II)

Cell-Wall Constituents of the Seed Coat.

(pp. 624～628)

By Syuiku SASAKI and Syôtarô Tô.

(Agricultural Chemical Laboratory, Kyûsyû Imperial University;
Received May 22, 1939.)

The soy-bean seed consists of 85～90% of embryo and 5～10% of seed coat. The present study relates to an investigation of the seed coat, especially to its cell-wall constituents.

Constituents of the Seed Coat.

	% of the seed coat (moisture free basis)
Pectin (0.5% am. oxalate extract)	17~20
Hemicelluloses (4% NaOH extract)	5.6~10.3
Crude fiber (Chlorination method)	51.9
α-Cellulose	31.2
β-Cellulose	18.2
γ-Cellulose	2.5
Crude protein	11.7
Crude oil	0.96
Ash	4.3

It will be observed from the above table that the main constituents of the seed coat are cellulose and pectin. The pectin could be isolated in a fairly pure state. The hemicelluloses were fractionated to the three types of hemicellulose A, hemicellulose B and hemicellulose C by means of acetic acid and acetone, and further purification was carried out with each fraction, but no pure substance was isolated.

Vol. 15 July 1939 No. 7

Bulletin of the Agricultural Chemical Society of Japan.

ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

Formation of β-Hydroxy-pyridine Derivatives from Hexoses and NH_3-salts. [I].

(pp. 629∼633)

By Kiyosi Aso.

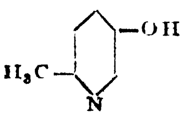
(Agricult. Chem. Lab., Tokyô Imp. Univ.; Received June 29, 1939.)

By heating the water solution of glucose or sucrose with NH_3-salts in an autoclave, the author obtained 3 different bases, which melt respectively at 124∼125°, 166∼167° and 201∼202°. Each base gives a blue colour with Folin-Denis phenol reagent but no colour with 2, 6-dichloroquinonechloroimide (Gibbs' reaction).

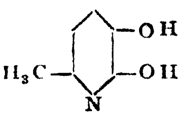
The base of m.p. 166∼167° C_6H_7NO crystallises in colourless prisms, giving a red colour with $FeCl_3$ and its picrate forms yellow prisms, melting at 203∼204°. This base was identified as 2-methyl-5-hydroxy-pyridine [I] since the free base as well as the picrate did not show depressed melting points on mixed tests with the corresponding authentic specimens.

The base of m.p. 201∼202° is obtained in silky crystals (formula: $C_6H_7NO_2$) and its water solution is neutral and gives a deep blue colour with $FeCl_3$ and does not form a picrate. The base has properties closely resembling 2- or 3-methyl-5, 6-dihydroxy-pyridine ([II] or [III]) synthesised by Feist. Although it is not yet proved by means of the mixed melting point, it may probably be the same as [II].

The base of m.p. 124∼125° is separated from ligroin-alcohol in colourless scaly plates (formula $C_6H_7NO_2$), and its water solution is neutral and gives a reddish brown colour with $FeCl_3$, and its picrate is obtained in yellow prisms melting at 182∼183°. Although its investigation is now being continued, the base may be perhaps 2-hydroxymethyl-5-hydroxy-pyridine [IV] which may be derived from hydroxymethyl-furfural [V] and NH_3 as next equation.

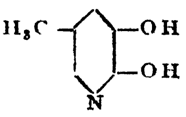


[I]

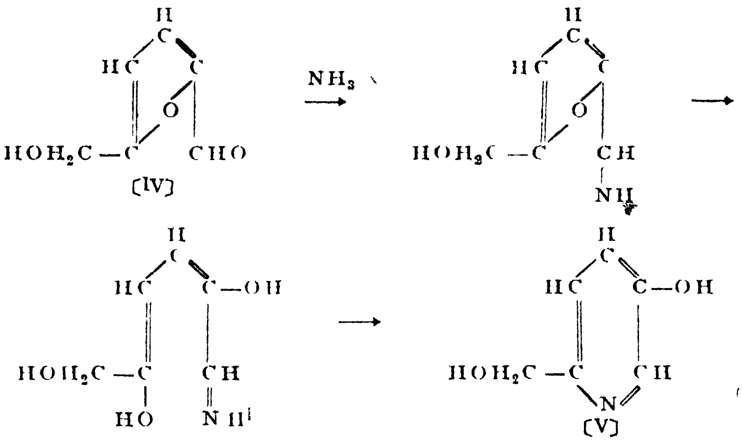


[II]

or



[III]



Studies on the Vegetable Tannins in Formosa.

(pp. 634~638)

By Yasuyosi Osima and Hirosi Iio
(Agricultural Chemical Department, Taihoku Imperial University,
Formosa; Received June 13, 1939)

Part 3.*

The tannin contents were determined by the international official method, and the chemical properties of tannins were studied qualitatively. The results were as follows:

Plant names	Water %	Soluble matter %	Non-tannin %	Tannin %	Classification %
Pithecolobium dulce Benth. (bark)	12.6	35.3	8.7	26.6	catechol
Casuarina equisetifolia Lin (″)	8.6	19.2	2.3	16.8	[illegible]
Ficus elastica Roxb. (″)	10.1	19.9	5.6	14.3	″
Bischoffia javanica Blume (″)	12.7	9.4	5.0	4.4	″
Elaeocarpus serratus Linn (″)	9.4	21.5	3.4	18.1	″
Liquidambar formosana Hance (″)	8.6	15.5	4.6	10.9	″
Elaeocarpus elliptica Nakai (″)	8.6	18.0	4.2	13.8	″
Ficus religiosa Line. (″)	8.6	13.5	4.8	8.7	″
Melaleuca leucadendron Linn. (wood)	8.4	3.5	1.0	2.4	″

The absorption amounts of tannins with hide powder were determined by the method reported in Part 2 of this paper. The results are shown in the following:

Tannins: *	pH						
	2	3	4	5	6	7	8
Pithecolobium dulce Benth	45.5	36.8	32.4	33.1	33.5	34.5	34.5
Casuarina equisetifolia Linn.	43.5	29.5	25.2	25.4	26.4	27.1	27.8
Ficus elastica Roxb.	43.4	27.9	23.6	21.6	22.6	23.6	24.3

* Bull. Agr. Chem. Soc. Japan, Vol. 15, 19, 39 (1939).

Part 4.

By Yasuyosi OSIMA.

A new catechin has been isolated from the bark of *Casuarina equisetifolia* Linn., named Mokumao in Formosa. It has been shown to be a stereoisomer of the gallo-catechin isolated from tea leaves (Bull. Agr. Chem. Soc. Japan, Vol. 12, No. 8, 1, 1936). This new substance was named "casuarin".

The chloroform solution obtained during the preparation of tannin (B) (Bull. Agr. Chem. Soc. Japan, Vol. 15, 39, 1939) was shaken with water and the water solution was concentrated under diminished pressure and cooled in an ice box. Then the casuarin had crystallised out and it was recrystallised from water. The principal properties are briefly shown in the following:

Physico-chemical properties		Colour reactions:	
m.p.	182°	$FeCl_3$	blue-green
$[\alpha]_D$ in aceton-water (1 : 1)	+19.7	Gelatin	no ppt.
$[\alpha]_D$ in alcohol	9	Lime water	purple
m.p. of hexacetate	127~128°	HCl-Formalin	White ppt.
$[\alpha]_D$ in aceton "	+34.3	Bromin water	Yellow ppt.
m.p. of penta-methylether	156~158°	KCN	red
$[\alpha]_D$ in acetone	+29.2	Pinewood-HCl	red

The reduction product with metallic sodium was identical with hexamethoxy-diphenyl-propan from gallo-catechin and with a synthetic specimen (loc. cit.). From these results, casuarin has been proved to have the same structure as gallo-catechin, but it is a stereoisomer and corresponds to *d*-form while gallo-catechin from tea is of l-epi-form.

Über die Spiritusfabrikation unter Verwendung von Dextrinvergärenden Hefen.

(pp. 639~644)

Von Shinji Doi.

(Aus der Agrikult. Chem. Labor., Kaiserl. Univ. zu Tokio;
Received June 7, 1939.)

Isolation of Four Kinds of Isoflavon from Soya Bean.

(pp. 645~652)

By Koji OKANO and Iwao BEPPU.

(The Central Laboratory of the South Manchurian Railway Co.,
Received June 29, 1939.)

We obtained the following isoflavons from the byproduct of the alcohol extraction of soya bean :—

(1) 8-methyl-5, 4′-dioxyisoflavon, mp. 318°C, white needle, named Tatoin.

(2) 8-methyl-5, 7, 4′-trioxyisoflavon, mp. 298°C, slightly yellow needle, named Methylgenistein.

(3) A glucoside of 8-methyl-5, 7, 2′-trioxyisoflavon, mp. 255°C, slightly yellow needle, named Methylisogenistin.

(4) A glucoside of 5, 7, 2′-trioxyisoflavon, mp. 265°C, also slightly yellow needle, named Isogenistin.

A Method for Measuring the Color of Fatty Oil.

(pp. 653~658)

By Dr. Kokichi OSHIMA and Tatsuro SUGAWARA.

(From the Chemical Laboratory of the Hakodate College of Fisheries, Japan,
Received June 5, 1939.)

Three sets of standard colors in sealed glass tubes were employed as follows :

Symbol and No.		Contents	Unit with Lovibond tintometer			
B (Brown)	1	10/3% $K_2Cr_2O_7$ + 2/3% $CrK(SO_4)_2 \cdot 12H_2O$	Yellow	637.3	Red	24.99
〃	2	1/2[10/3% $K_2Cr_2O_7$ + 2/3% $CrK(SO_4)_2 \cdot 12H_2O$	〃	297.4	〃	11.66
〃	3	1/4[〃]	〃	148.7	〃	5.83
〃	4	1/8[〃]	〃	69.4	〃	2.72
〃	5	1/16[〃]	〃	37.0	〃	1.45
〃	6	1/32[〃]	〃	19.7	〃	1.00
〃	7	1/64[〃]	〃	8.0	〃	0.60
〃	8	1/128[〃]	〃	3.0	〃	0.48
〃	9	1/256[〃]	〃	1.3	〃	0.30
〃	10	1/512[〃]	〃	0.4	〃	0.14
〃	11	1/1024[〃]	〃	0.25	〃	0.08
〃	12	1/2048[〃]	〃	0.13	〃	0.04
〃	13	1/4096[〃]	〃	0.07	〃	0.02
Y (Yellow)	1	8% K_2CrO_4	〃	175.8		
〃	2	4% 〃	〃	105.5		
〃	3	2% 〃	〃	59.8		
〃	4	1% 〃	〃	37.9		
〃	5	1/2% 〃	〃	18.0		

″	6	1/4%	″	″	13.0		
″	7	1/8%	″	″	7.8		
″	8	1/16%	″	″	4.8		
″	9	1/32%	″	″	2.6		
″	10	1/64%	″	″	1.4		
″	11	1/128%	″	″	0.69		
″	12	1/256%	″	″	0.40		
″	13	1/512%	″	″	0.22		
R (Red)	1	20%	$Co(NO_3)_2 \cdot 6H_2O$	″	1.15	Red	17.0
″	2	10%	″	″	0.7	″	10.8
″	3	5%	″	″	0.4	″	6.4
″	4	5/2%	″	″	0.2	″	3.5
″	5	5/4%	″	″	0	″	1.75
″	6	5/8%	″	″	0	″	0.9
″	7	5/16%	″	″	0	″	0.6
″	8	5/32%	″	″	0	″	0.32

A sample of fatty oil is placed in a test tube which is the same size as those of the standard colors and is compared with the standard color tubes in a comparator box. The result of the comparison is expressed by the symbol and number of the standard tube whose color is identical with that of the sample oil.

The colors of the standard solutions do not fade for a long period of time even under direct sunlight.

On the Stimulant for Cane Sugar Formation in Plants. (III)

(pp. 659∼660)

By Tetutaro TADOKORO.

(Biochem. Lab., Faculty of Sci., Hokkaido Imp. Univ.,
Received June 15, 1939)

Studies on the Amylo-Process for the Production of Alcohol from Potatoes. (II).

(pp. 661∼668)

By Shinji DOI.

(Agricultural Chemical Laboratory, Tokyo Imperial University;
Received June 7, 1939.)

Statistic Studies of Soils. (VIII).

(pp. 669~684)

By Dr. Misu-Hideo and Rihoki.

(Agricultural Experiment Station, Government General of Tyôsen;
Received June 2, 1939.)

Synthesis of Iso-ochracin.

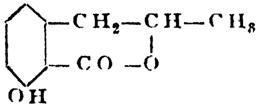
The Metabolic Products of *Aspergillus Ochraceus*. Part III.

(pp. 685~689)

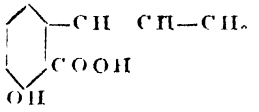
By Teiichi Tamura.

(Agricult. Chem. Lab., Tokyô Imp. Univ; Received June 29, 1939.)

It has been shown by T. Yabuta and Y. Sumiki that ochracin (I), a new metabolic product of *Aspergillus ochraceus*, yielded melleic acid (II) on kalifusion under a mild condition, from which, by treatment with sulphuric acid, iso-ochracin (III) was prepared.



(I)



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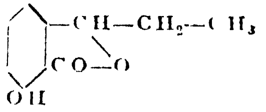
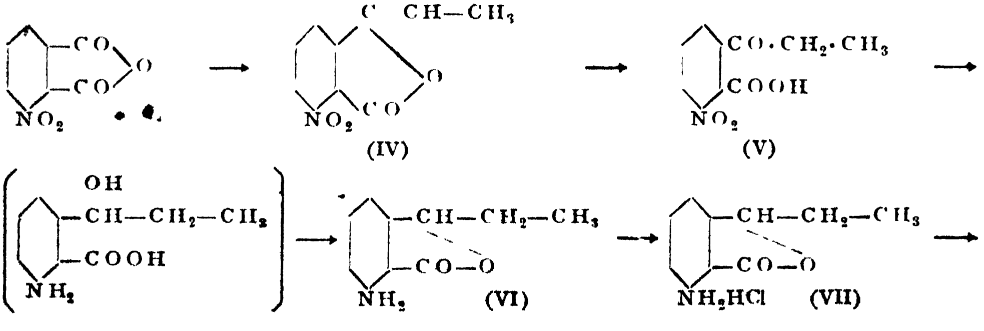
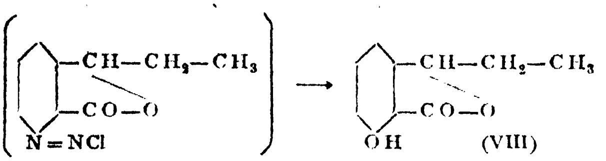


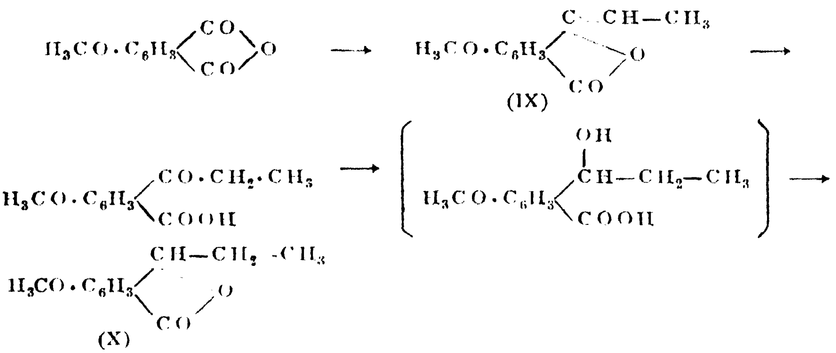
(III)

The author has now confirmed by the following synthesis that iso-ochracin is identical with 7-oxy-ethylphthalide substantiating the conclusion of Yabuta and Sumiki. According to Gabriel's method for preparing benzalphthalides, 3-nitrophthalic anhydride is heated with propionic anhydride and anhydrous sodium propionate to prepare 7-nitro-ethyliden-phthalide (IV). IV is dissolved in warm dilute alkali and 6-nitro-2-propionyl-benzoic acid (V) is obtained. By the reduction of V with a large excess of sodium amalgam, 2^1-oxy-2-propyl-6-amino-benzoic acid is obtained, which, when neutralized with hydrochloric acid, gives 7-amino-ethylphthalide (VI). From (VI) by treating with hydrochloric acid in alcoholic solution, 7-amino-ethylphthalide chlorohydrate (VII) is obtained. This substance is diazotized and led to 7-oxy-ethylphthalide (VIII), identical in all respects with iso-ochracin.





In the next experiment, synthesis of methyl-iso-ochracin was attemped, starting from 3-methoxy-phthalic anhydride instead of from 3-nitrophthalic anhydride. According to the same method above described, 7- or 4-methoxy-ethyliden phthalide (IX) is prepared. This substance, on reduction with sodium-amalgam and on subsequent acidification with sulphuric acid, gives 7- or 4-methoxy-ethyl-phthalide (X), m.p. 58°.



On the contrary, the methylated product of iso-ochracin which is prepared by its methylation with diazomethane, dimethylsulfate or methyliodide is obtained only as an oily matter.

From this experiment, the resulting crystal (X) is recognized as 4-methoxy ethylphthalide to the best of my belief, although the choice between the 4- or 7-isomer is not yet clearly decided.

Chemical Studies on the Kikyo-root. (Report VII).

The molecular weight and the hydrolysis of platycodin.

(pp. 690~695)

By M. Tsujimoto and T. Matsumoto.

(Agr. Chem. Laboratory, Kyushu Imp. Univ., and Agr. Chem. Laboratory, Imp. College of Agr. and Forestry; Received June 30, 1939)

Conclusions

(1) The result of the elementary analysis of platycodin was as follows:

Found	C%=60.11	H%=8.06
Calc. for $C_{43}H_{68}O_{17}$	〃 =59.72	〃 =8.06

(2) The molecular weight of platycodin was determined by the method of titration and Barger's method.

Titration method (as monobasic acid)	836
Barger's method	801
Mean	819
Calc. for $C_{42}H_{68}O_{17}$	844

(3) The following two processes of hydrolysis of platycodin were examined.

a. By boiling it with 5%-alcoholic HCl the hydrolysis was completed in 20 hours.

b. By boiling it with 5%-alcoholic H_2SO_4 the hydrolysis was completed in 100 hours.

(4) The qualitative determination of sugar in the syrup.

a. Pentose	Negative	e. Galactose	Negative
b. Methyl pentose	〃	f. Mannose	〃
c. Glucuronic acid	〃	g. Glucose	Positive
d. Keto-hexose	〃	h. Tiglic acid	Negative

Therefore, the sugar produced by the hydrolysis of platycodin is glucose only.

(5) From the above, the hydrolysis of platycodin by acids may be represented by the following equation.

$$\underset{\text{Platycodin}}{C_{42}H_{68}O_{17}}+2H_2O=\underset{\text{Platycodigenin}}{C_{30}H_{48}O_7}+\underset{\text{Glucose}}{2C_6H_{12}O_6}$$

Vol. 15 August 1939 No. 8

Bulletin of the Agricultural Chemical Society of Japan.

ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

On the Application of Hydrogen Peroxide for Brewing.

(pp. 699~710)

(Part I: See Bull. Agricult. Chem. Soc. Jap., Vol. 13, 59~60, 1938.)

By Masakazu YAMADA.

(The Governmental Institute of Brewing, Takinogawa, Tokyo;
Received July 19, 1939.)

Part II. On the catalase action at the various stages in saké brewing.

The catalase action at the various stages in saké brewing becomes maximum in the water extract of kozi and then decreases gradually according to the progress of stages—moto-mash, moromi-mash and saké in order. It declines suddenly by pasteurization. When saké putrefies, the catalase action grows weak.

EXPERIMENTAL.

The estimation of catalase: To 2~10 cc of enzyme solution add 2~5 cc of 1% H_2O_2 and stand for 2 hours in the ordinary temperature. Then add 10 cc of 4 % KI solution and 10 cc of 5% H_2SO_4. After 1 hour brown colored solution is titrated with $N/10$ $Na_2S_2O_3$, using starch solution as the indicator.

Grs. of H_2O_2 decomposed by 100 cc of enzyme solution for 2 hours in the ordinary temperature were as follows:—

I. Kozi extract.

Kozi was extracted with the same volume of water.

1. 10% of the surface of the boiled rice grain was covered with mycel: 0.0807 g.
2. 30% of the surface 〃 : 0.1363 g.
3. 70% of the surface 〃 : 0.6546 g.
4. The entire surface 〃 : 1.2016 g.

II. Moto mash. (filtrate)

1. The beginning of the alcoholic fermentation: 0.4050 g, 0.3174 g.
2. Moto mash completed: 0.1973 g, 0.1853 g.

III. Moromi mash. (filtrate)

1. At the 12th day: 0.1238 g.
2. Moromi mash completed: 0.0915 g.

IV. Fresh saké.

1. Before pasteurization: 0.0352 g∼0.1744 g (50 samples)
2. The variation after pasteurization:

	Apr. 15						
No. of samples of saké	before pasteur.	after pasteur.	Jun. 23	Aug. 6	Sept. 8	Oct. 7	Nov. 7
2	0.0520 g	0.0402	0.0392	0.0235	—	—	—
3	0.0855	0.0604	0.0629	0.0641	0.0547	0.0484	0.0456
10	0.1073	0.0486	0.0410	0.0486	0.0409	0.0270	0.0242

V. The putrefaction of saké.

Saké was diluted with one fifth part of water and bottled. To A a drop of putrefied saké was added.

	after 2 days	after 5 days	after 8 days	after 12 days
A.	0.0772 (cloudy)	0.0629	0.0475 (turbid)	0.0404
B. (control)	0.0748	0.0605	0.0516	0.0582

By Masakazu YAMADA and Syōzō MASUI.

Part III. On the influence of alcohol and temperature upon the catalase of saké.

The catalase in saké is not destroyed by pasteurization but remains more or less in old saké, while the diastase is completely destroyed by heating to ca. 45∼60°C, which are the usual temperatures of pasteurization for saké. In this case ca. 17% of alcohol content is quite significant. (See this journal Vol. 11. 28∼9. 1935) Our experiment showed that the catalase in kozi extract was first destroyed by heating to 80°C, while the catalase in saké was destroyed by heating to 70°C.

In this case also, the existence of alcohol strengthens the destructive power of heating.

By Hisao MATUI and Masakazu YAMADA.

Part IV. On the destruction of the catalase in saké by hydrogen peroxide.

As we reported in a previous paper (this bulletin Vol. 13, 59~60, 1938.), the unpleasant "hioti" smell of putrefied saké can be removed by hydrogen peroxide. It was found that saké did not putrefy when it contained a little quantity of hydrogen peroxide. (See Part VII.) But if hydrogen peroxide is directly added to saké, it is unavailably decomposed, for saké contains catalase. Therefore we must first destroy the catalase in saké, when we want to add hydrogen peroxide to saké for the above mentioned purposes.

Our experiments showed that one thousandth part of 35% hydrogen peroxide added to saké was completely decomposed in 24 hours by catalase. Indeed hydrogen peroxide has destructive action on catalase, but four thousandths part of 35% hydrogen peroxide is required in order to destroy the catalase in saké. On the other hand catalase is inactivated by heating. Generally catalase is not entirely destroyed at 70°C, but in the case of saké the catalase is completely destroyed at that temperature owing to the aid of alcohol content (15~18%).

The method of destroying catalase as stated above needs hydrogen peroxide, which is too wasteful, and too high temperature for practical use, and so we must treat saké as follows to overcome the difficulty: Heat saké at about 50°C and add one thousandth part of 35% hydrogen peroxide at this temperature, the catalase in saké being thus inactivated. In this case also alcohol reveals the destructive action for the enzyme at a lower temperature.

By Hisao MATUI and Masakazu YAMADA.

Part V. The action of hydrogen peroxide on diacetyl, acetoin and butylene glycol.

In a previous paper (this bulletin vol. 13, 59~60, 1938.) we reported that in the putrefied saké both diacetyl and acetoin increased and the former was oxidised by hydrogen peroxide to acetic acid. Now it has been ascertained that when hydrogen peroxide is added in order to remove the bad smell of the putrefied saké, the chemical does not react with both acetoin and its correlative substance 2·3 butylene glycol.

Statistic Studies of Soils. (IX).

(pp. 710~722)

By Dr. MISU-Hideo and RIHOKI.

(Agricultural Experiment Station, Government General of Tyôsen;
Received June 2, 1939.)

Nutritional Chemistry of Flowers. (Part III)

Protein in Wisteria Flowers.

(Kraunhia floribunda Taub. var. typica Mak.)

(pp. 723~726)

By Sakae SHINANO.

(Laboratory of Nutritional Chemistry, Kyoto Imp. University;
Received July, 14, 1939.)

A kind of conjugated protein was isolated from the dilute-NaOH-extracts of dried wisteria flowers and its nature examined.

1) It was completely precipitated at p_HH 4.16.

2) The nitrogen content was 10.51~10.27%, and the ash content 2.85~2.43% in the water-free protein.

3) The conjugated protein was hydrolyzed and the nitrogen distribution determined by the routine method.

The result showed a high content of HCl-insoluble nitrogen, a low humin-N content, and a generous amount of whole nitrogen in the basic amino acids, especially in lysin-N and cystin-N.

4) The percentages of phosphorus, iron, and copper in the water-free protein were determined, the results being as follows;

P: 0.170%, Fe: 0.082%, Cu: 0.101%.

5) The conjugated protein was denatured during the course of the experiment by dialysis and treatment with alcohol and ether.

Owing to its being denatured the greater part of the conjugated protein was insoluble in dilute NaOH.

6) In that part which was soluble in dilute NaOH (0.04 *N*) the nitrogen content was small, while on the contrary, the carbohydrate content was great.

(1939, April 2)

The Fractionation of the Soy-bean Protein.

(pp. 727~736)

By Kinsuke KONDO, Seiichi HAYASHI and Shinichi MORISHIGE.

(Laboratory of the Nutritional Chemistry, Dep. of Agriculture and Chemical Institute, Kyoto Imperial University;
Received July 14, 1939.)

Studies on the Absorption-spectrum of the Vitellin prepared from the Yolk of Logger-head Turtle Eggs.

(pp. 737~743)

By Kinsuke KONDO, Masao NAGASHIMA and Shinichi MORISHIGE.

(Laboratory of the Nutritional Chemistry, Dep. of Agriculture and Chemical Institute, Kyoto Imperial University; Received July 14, 1939.)

On the Soil Reaction and Lime Status of Apple Orchard.

(pp. 744~750)

By R. KAWASHIMA.

(Agr. Chem. Laboratory, Kyushu Imp. University; Received July 17, 1939.)

The apple orchard soils examined have very varying degrees of reaction and lime saturation status. But the soils of productive orchards tend to have pH values ranging 5.5~6.0, and about fifty per cent lime saturation.

Über die auf Pilzdecke Entstehende Kristallsubstanz bei der Oxalsäuregärung.

(ss. 751~752)

Von M. SIMO.

(The Government Industrial Reserch Institute, Taiwan, Nippon;
Received July 17, 1939.)

The Optimum Hydrogen Ion Concentration for Amylase of *Aspergillus Oryzae*.

(pp. 753~756)

By Takeo NAGATOMO.

(Mirin-Department of Noda-Shoyu Co.; Received July 22, 1939.)

Der Einfluss des Kalis auf den Kohlenhydratgehalt in den Blättern und Halmen der Reispflanzen.

(ss. 757~767)

Von Tatsuo KOYANAGI.

(The Imperial Sericultural Experiment Station; Received July 3, 1939.)

Um ein Bild davon zu erhalten, welchen Einfluss die verschiedenen Kaligaben auf den Kohlenhydratgehalt in den Blättern und Halmen der Reispflanzen auszuüben vermögen, habe ich in den letzten Jahren einige Gefässversuche durchgeführt.

I. Versuch: Eine Reihe von Wagnerschen Vegetationsgefässen wurde mit „Nisigahara“-Boden (einem diluvialen Boden) und eine andere Reihe mit „Arakawa“-Boden (einem alluvialen Boden) gefüllt, und zu jedem Topf wurden die verschiedenen Düngermengen (Siehe Tabelle I im Text) zugesetzt. Ende Juni 1933 wurden die gleichgross zugewachsenen jungen Reispflanzen herausgesucht und eingepflanzt. Die Pflanzen wurden während des Vegetationsstadiums periodisch herausgepflückt, und es wurden darin das Kohlenhydrat und einige, andere Bestandteile nach den folgenden Methoden bestimmt.

Das Analysenmaterial wurde am Mittag bei klarem Wetter gesammelt und nach der Linkschen Methode (9) getrocknet. Das pulverisierte Material wurde nach dem Davis u. Daishschen Verfahren (10) mit Alkohol ausgezogen und der Zucker im Filtrat nach der Hydrolyse durch die Hagedorn u. Jensensche Methode (12) bestimmt. Der Rückstand des Zuckerauszugs wurde zur Stärkebestimmung durch das Oshima u. Itayasche Takadiastase-Verfahren (13) benutzt. Die Tabelle II fasst diese Analysenergebnisse und das Gewicht der Ernteprodukte zusammen.

II. Versuch: Der Versuch wurde im Jahre 1934 wiederholt. Die im vorigen Versuche angewandten Böden der vier Parzellen wurden mit Sand bis auf 70% durchgemischt und zur neuen Plantage wurde Kalidünger in einer etwas steiler zunehmenden Weise (Tabelle III) zugesetzt. Die sonstige Versuchsanordnung blieb gleich wie die vorige. Die Analysenmethode unterscheidt sich aber von der vorigen dadurch, dass, man zum Zweck der Bestimmung des einfachen Zuckers das Material gleich nach der Ernte direkt mit heissem Alkohol auszog und die reduzierende Substanz vor der Hydrolyse als einfachen Zucker bestimmte. Tabelle IV fasst die Gesamtergebnisse des zweiten Versuche zusammen.

Aus diesen zwei Versuchen ergab sich, dass sich der Zuckergehalt in den Blättern von Anfang Juli bis Anfang August mit der steigenden Kaligabe vermindert, der Stärkegehalt dagegen vermehrt. Diese Ergebnisse stimmen zwar in bezug auf den Zuckergehalt mit den Befunden von Janssen u. Bartholomew (6), Nightingale (7) und Hartt (8) überein, die den Zucker in den Blättern und Halmen, der an Kali mangelnden Sojabohnen, Tomaten und Zuckerröhre reichlich fanden; aber sie stimmen nicht in bezug auf den Stärkegehalt mit den Befunden von Burrel (14) und Hartwell (15) überein, die den erhöhten Stärkegehalt in den Blättern und Halmen der an Kali mangelnden Sojabohnen, Tomaten und Kartoffeln angaben.

Die Ende August (kurz von der Zeit des Schiessens in Ähren) zeigten die geernteten Pflanzen unter variierten Kaligaben einen ganz anderen Kohlenhydratspiegel als die jüngeren Pflanzen. Die mässig mit Kali gedüngten Pflanzen waren nicht nur durch ihren hohen Zucker- und Stärkegehalt, sondern auch durch die Grösse ihrer Ernteprodukte ausgezeichnet. Eine ähnliche Tatsache beobachteten Janssen und Bartholomew (18) bei der Wasserkultur von Buchweizen, Mais und

Klee während ihrer Blütezeit, bzw. der Zeit ihrer Reife und nahmen daraus an, dass die Neubilduhg des Kohlenhydrats nicht der Kaliaufnahme proportionell, sondern nur bei der mässigen Kaligabe maximal ist.

Nach der Blütezeit dagegen wurde der Einfluss der Kaligabe auf den Kohlenhydratgehalt in den einzelnen Blättern und Halmen der Reispflanzen so unregelmässig, dass sich der Zusammenhang der Kalidüngung mit dem Kohlehydratgehalt aus meinem Versuch nicht feststellen liess.

Durch steigende Kaligaben wurde der Stickstoffgehalt in den Blättern und Halmen während des ganzen Wachstums meist etwas herabgedrückt. Überdies ist eine ähnliche Tatsache schon bei anderen Pflanzen z. B. Sojabohne, Tomate und Buchweizen von den oben zitierten Forschern (14) (7) (6) (16) berichtet worden. Beim erhöhten Kalizusatz zum „Arakawa“-Boden trat eine Ertragsabnahme ein. Selbst in solchem gestörten Wachstum war der Stickstoffgehalt immer weniger als normal. Dabei wurde also die gesamte Menge des von den Pflanzen absorbierten Stickstoffs herabgesetzt. Daraus lässt sich annehmen, dass der Kalizusatz höchst wahrscheinlich die Stickstoffaufnahme der Wurzel hemmt. Diese hemmende Wirkung des Kalis auf die Stickstoffresorption stellten auch Mevius u. Dikussar (19) aus ihrem Wasserkulturversuch der Sojabohnen vor.

Auf Grund der Ergebnisse meiner Gefässversuche steht es zweifellos fest, dass der Kohlenhydrat- und Stickstoffgehalt in sehr inniger Beziehung zueinander stehen.

III. Versuch: Nun kommt eine weiteren Frage, in welcher Weise der Kohlenhydratspiegel bei variierender Stickstoffgabe durch Kalizusatz modifiziert wird. Zur Lösung dieses Problems wurde eine Reihe von Gefässversuchen mit den jüngeren Pflanzen vorgeführt, bei denen sowie Kali auch Stickstoff in verschiedenen Mengenverhältnissen zugesetzt wurde. Der „Nisigahara“-Boden, worauf man ohne Kalidüngung schon dreimal geerntet hatte, wurde wie in II. Versuche mit Sand bis auf 70% durchgemischt und dazu mit dem Dünger in verschiedenen Mengen versetzt. Diese Ergebnisse (Siehe Tabelle V) erwiesen, dass in Gegenwart von 1.5 und 3.0 g Stickstoff in jedem Gefässe die Kali-freie Gruppe an Zucker reicher als die mit Kali gedüngter Gruppe ist, also blieb das Verhältnis ganz gleich wie beim vorigen Versuch. Im Falle des Mangels an Stickstoffdünger führte die Kali-freie Ernährung die Herabsetzung des Stärke- und Zuckergehaltes herbei. Bei der gleichen Kalizufuhr ist der Zuckergehalt der Versuchspflanzen von dem Grad des Stickstoffshungers abhängig.

IV. Versuch: Es ist eine Frage, ob das in dem I. und II. Versuche gefundene Verhältnis zwischen Kalidüngung und dem Kohlenhydratgehalt in den Pflanzen den ganzen Tag unverändert bleibt, weil der Kohlenhydratgehalt einer ziemlich starken Tagesschwankung zu unterliegen scheint. Zur Klärung dieser Frage wurde eine Reihe von Versuchen im Freiland angestellt. Die Grösse jeder Parzelle betrug 3 x 1.5 m. Von den Parzellen mit- und ohne-Kalidüngung, auf denen ich vier Jahr hindurch die, wie in Tabelle VI angegeben, gedüngten Reispflanzen geerntet hatte, sammelte ich das Analysenmaterial viermal an einem Tag und bestimmte den Zuckergehalt darin nach der im II. Versuch berichteten Methode, die Stickstoffssorten wurden nach Mothes (20) bestimmt. Die in der Tabelle VII zusammengefassten Ergebnisse zeigen, dass die Pflanzen auf den Parzellen ohne Kali Tag und Nacht hindurch den Zucker immer in grösseren Mengen enthielten, als die auf den Parzellen ohne Kali, während betreffs des Stärkegehaltes dieses Verhältnis gerade umgekehrt war. Aus der Tabelle geht hervor, dass die Kalidüngung eine Erhöhung des Wassergehaltes und eine Herabsetzung des Amidostickstoffs bewirkte.

Die Tabelle VIII gibt eine Übersicht des ersten und zweiten Versuches und des Vorversuches, der in 1932 durchgeführt wurde.

Beim Betrachten dieser Übersichtstabelle ist zu bemerken, dass die überschüssige Kalizufuhr gewissermassen die Ernte herabsetzt, und ferner, dass meine Ergebnisse in grossen Zügen den Angaben von Janssen u. Bartholomew (18) bzw. Gassner (21) (22) entsprechen. Die ersteren sahen bei der Wasserkultur des Hafers die Trockensubstanz bei mässiger Kaligabe zur maximalen Neubildung kommen und der letztere beobachtete bei der Sandkultur der jungen Gramineen bei erhöhter Kaligabe eine deutliche Senkung der Kohlensäureassimilation.

Aus meinen Untersuchungen lässt sich schliessen, dass der Kalidünger zwar bis zu einem gewissen Grade das Wachstum der Reispflanzen günstig beeinflusst, aber bei seinem Überschuss die Stickstoffabsorption und Kohlensäureassimilation gehemmt werden und infolgedessen eine Ertragsverminderung verursacht wird.

Die ganze Reihe von Versuchen wurde in der Kaiserlichen Landwirtschaftlichen Versuchsstation Nisigahara, Tokio unter der Leitung von Herrn Dr. M. Shioiri durchgeführt.

On the Method of Quantitative Estimation of Glutathione in Tissue.

(pp. 758～774)

By Masayoshi OGAWA.

(Department of Nutrition, College of Medicine, Nippon University;
Received July 28, 1939.)

The Effect of Glutathione on Fever and Glycolysis.

(Biochemical Studies on Glutathione. The Xth Report.)

(pp. 775～782)

By Masayoshi OGAWA.

(Department of Nutrition, College of Medicine, Nippon University;
Received July 28, 1939.)

In the previous communication the author reported the effect of glutathione

upon fever.

In the present report, he performed an experiment on the effect of GSH upon fever and glycolysis, employing several rabbits which became feverish through hyperglycemia produced by intravenous glucose injections.

Okuda-Ogawa's micro method for the determination of glutathione in HPO_3 extract of blood and Hagedorn-Jensen's method for the determination of blood sugar were employed and the following results were obtained:

I) The blood sugar and body temperature of the control animals began to rise and reaching their maximum 2 and 3 hours after the injection of glucose, they subsided to the normal level within 5 and 6 hours.

II) In the animals to which 0.1, 0.5, 1.5, and 5.0 mg of glutathione (GSH) per kg body weight were administered intravenously, blood sugar abated within the first 5.0, 3.1/2, 3.0, and 2.1/2 hours respectively, although the body temperature subsided to the normal level during the next 1.0, 1.0, 1.0, and 1/2 hours.

III) Through-out the experiment no influence upon the blood glutathione (GSH, GS-SG) was observed.

Note on Okuda's Iodine Method for the Determination of Cystine.

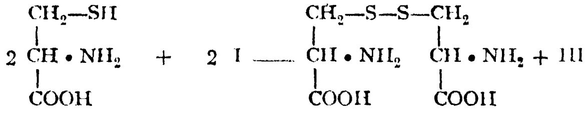
(pp. 783～790)

By Masakazu SATO, Tamotu HIRANO and Miss TANGETU Kan.

(Laboratory of Nutrition Chemistry, Taihoku Imperial University, Formosa, Japan;
Received July 27, 1939.)

The results obtained are summarized below:—

I. In Okuda's method, we found the conditions under which the reaction between the cysteine and iodine proceeds according to the following chemical equation,



$$2\,\begin{matrix}CH_2-SH\\|\\CH\cdot NH_2\\|\\COOH\end{matrix} + 2\,I \longrightarrow \begin{matrix}CH_2-S-S-CH_2\\|\qquad\qquad|\\CH\cdot NH_2\quad CH\cdot NH_2\\|\qquad\qquad|\\COOH\qquad COOH\end{matrix} + HI$$

i. e., Cysteine: I = 1 : 1.

The conditions were satisfied when the concentration of hydrochloric acid was 0.5～1 *n*, the temperature of the solution to be titrated 0°～8°C and the concentration of potassium iodide 0.01～0.03 mol.

II. We re-examined further the several processes in this method and found in addition several points to be improved:—

1) With regard to the reduction process of cystine, we found that there is a close co-relation among the quantity of zinc, the concentration of the acid, the temperature at the time of reduction, the time required for the reduction and the

quantity of the cystine taken. According to our experiments, 30 minutes are sufficient when the reduction was carried out at 20°C or at the temperature higher than that, using 2 g of zinc and 60 cc of 1 *n* hydrochloric acid corresponding to 0.1 mg mol of cystine.

2) As the washing solvent to be used after the reduction as well as after the de-coloration, 0.5 *n* hydrochloric acid was preferred to hot water which had generally been used.

3) Special attention must be paid when the acidity of the solution to be titrated has to be regulated by the addition of alkali solution, because there is a danger that the cysteine might partially be decomposed. In this case it was found to be better to regulate the acidity by the addition of water.

4) Some remarks were also made on the addition of the starch solution as an indicator, on the treatment of the reagents solution used, as well as on the order of the addition of reagents, etc.

III. On the basis of these experimental results (I and II) we have made many improvements on Okuda's method.

On the Amylalcohol employed in the Van Slyke Method for the Determination of the Distribution of Nitrogen of Proteins.

(pp. 791∼794)

By Masakazu SATO, Tamotu HIRANO and Hirosi KAMATI.

(From the Laboratory of Nutrition Chemistry, Faculty of Science and Agriculture, Taihoku Imperial University, Formosa, Japan; Received July 27, 1939.)

In the determination of the distribution of nitrogen of proteins by the Van Slyke method, considerably great errors beyond experimental were sometimes obtained in the part of the basic amino acids fraction. Therefore, it was undertaken by the authors to search out the source of these errors and finally it was found to be due to the pyridine which was sometimes contained as one of the impurities in the amylalcohol employed. (The pyridine contents of five commercial amylalcohol preparations were determined as 0.858, 0.147, 0.045, 0 and 0 % respectively).

In the case of using amylalcohol which contains a certain amount of pyridine, it was found that the pyridine remains in the basic amino acids fraction in the form of its salt with hydrochloric acid and becomes the source of the errors owing to the following reasons;

1. In the case of determining the total nitrogen of the diamino acids fraction by the Kjeldahl method, the decomposition of pyridine by the concentrated sulphuric acid is considerably diffcult and might remain as its salt with sulphuric acid unless special attention is paid in the method of its decomposition. The pyridine thus remaining is again made free and comes into the distillate on the

distillation process and has no small influence upon the color change of the indicator, thus causing an error in titration. When special attention was paid in the method of decomposition of pyridine as previously mentioned and made its decomposition complete, the pyridine N should be added to the N of the basic amino acids fraction, thus causing, even in this case, an error of giving excessive value for the nitrogen.

2. In the case of determining the arginine N of the basic amino acid fraction, the pyridine is also made free as the fraction is made strongly alkaline, and comes into the distillate on the distillation process and has an influence similar to the above.

3. Consequently, the value of the histidine N as well as that of the lysine N would not be correct as these values are to be calculated from those of the total N and arginine N of the basic amino acid fraction.

For the above reasons, the authors wish to emphasise that the amylalcohol employed in the Van Slyke method should previously be freed from pyridine by means of shaking thrice with two volumes of 5 % HCl in a separating funnel.

Zuckerrohrwachs. IV. Mitteilung.

Über die Diol-derivate des Zuckerrohrsitosterins und des Stigmasterins.

(ss. 795~804)

Von Tetuo Mitui.

(Aus dem Agrikulturchem. Laboratorium der Kaiserl. Universität Kyoto;
Eingegangen am 31. Juni, 1939.)

In der III. Mitteilung berichtete ich, dass ich aus den unverseifbaren Substanzen von Zuckerrohrwachs zwei neue Bestandteile in kleinen Mengen gewonnen und sie α-Saccharostandiol bzw. β-Saccharostenon genannt habe. α-Saccharostandiol gehört zu einem gesättigten Steroid von der Formel $C_{29}H_{50}(OH)_2$. Hieraus habe ich vermutet, dass das α-Saccharostandiol ein Derivat von Stigmasterin oder Sitosterin ist, da α-Saccharostandiol die gleiche Gliederzahl des Kohlenstoffatoms wie Stigmasterin oder Sitosterin zeigt, und ausserdem im Zuckerrohrwachs sehr reichlich Stigmasterin und Sitosterin erhalten werden.

Hiermit habe ich aus dem Zuckerrohrsitosterin und dem Stigmasterin die folgenden gesättigten Diol-derivate synthetisiert.

1) Sitostan-3, 4-diol 199°
2) Sitostan-3, 6-diol 215°
3) Sitostan-3, 7-diol 176°
4) Stigmastan-3, 4-diol 200°
5) Stigmastan-3, 6-diol 213°
6) Stigmastan-3, 7-diol 174°

2), 3), 5) und 6) sind bisher in der Literatur noch nicht bekannte Substanzen. Wie man aus dieser Tabelle ersehen kann, ist α-Saccharostandiol nicht identisch mit diesen Diol-derivaten. Aber ich habe gefunden, dass die beiden Diol-derivate, die an der gleichen Stelle der Kohlenstoffatome zwei OH-Gruppen besitzen, den gleichen Schmelzpunkt und die gleiche Formel haben, und bei der Mischschmelzpunktprobe keine Depression zeigen. Ferner ist anzunehmen, dass das Zuckerrohrsitosterin dem 22-Dihydrostigmasterin identisch ist, und folglich seine Seitenkette nicht $-CH(CH_3)CH_2CH(CH_3)\ CH(CH_3)CH(CH_3)_2$ ist wie ich in der II. Mitteilung vermutete, sondern $-CH(CH_3)CH_2CH_2\ CH(C_2H_5)CH(CH_3)_2$ haben soll.

Diesen Widerspruch soll ich in der nachsten Mitteilung klar gemacht werden.

— — — —

Zuckerrohrwachs. V. Mitteilung.

Oxydationsversuche mit Zuckerrohrsitostanylacetat.

(ss. 805~808)

Von Tetuo MITUI.

(Aus dem Agrikulturchem. Laboratorium der Kaiserl. Universität Kyoto;
Eingegangen am 31. Juni, 1939.)

In meiner II. Mitteilung(3) berichtete ich, dass es mir gelungen ist, durch die Chromsaureoxydation des Zuckerrohrsitosterins 3-Oxy-bisnor-cholensaure zu gewinnen, und aus dem Ergebnis dieser Oxydation die Struktur der Seitenkette von Zuckerrohrsitosterin als $-CH(CH_3)CH_2CH(CH_3)CH(CH_3)CH(CH_3)_2$ zu vermuten. In der IV.(4) Mitteilung habe ich aber gefunden, dass die Struktur der Seitenkette $-CH(CH_3)CH_2CH_2CH(C_2H_5)CH(CH_3)_2$ sein soll.

Um diesen Widerspruch zu erklaren, versuchte ich den Oxydationsabbau des Zuckerrohrsitostanylacetats (Dihydrositosterylacetat). Mit Hilfe des Oxydationsverfahrens mittels Chromsaure konnte ich aus Zuckerrohrsitostanylacetat drei wichtige Derivate gewinnen, namlich trans-Androsteron, 3-Oxy-nor-allo-cholansaure und 3-Oxy-atio-allo-biliansaure. Die Schmelzpunkte dieser Stoffe und ihrer Derivate sind:

	Nach d. Verfasser (unkorr.)	Nach Literatur (korr.)
trans-Androsteron-acetat-semicarbazon	261~262°	262°(2)
3-Oxy-nor-allo-cholansäure	221~222°	223~226°(1)
〃 -methylester	154°	156°(1)
〃 -methylesteracetat	158°	163°(1)
3-Oxy-ätio-allo-biliansäure	238°	239°(5)
〃 -dimethylester	78° (74° sint.)	—

Die Tatsache, dass aus Zuckerrohrsitostanylacetat als Abbauprodukt 3-Oxy-nor-allo-cholansaure erhalten wurde, zeigt dass Zuckerrohrsitosterin wahrscheinlich 22-Dihydrostigmasterin ist.

LITERATUR.

(1) E. Fernholz: Ber., 67, 2021 (1934).
(2) L. Ruzicka: Helv., 18, 430 (1935).
(3) T. Mitui: Bull. Agricult. Chem. Soc. Japan, 14, 24 (1938).
(4) T. Mitui: Bull. Agricult. Chem. Soc. Japan, 15, 125 (1939).
(5) S. Kuwada: J. Pharm. Soc. Japan, 56, 631 (1936).

Vol. 15 September 1939 No. 9

Bulletin of the Agricultural Chemical Society of Japan.

ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

A Saline Alkali Soil in Manchuria.

(pp. 809~814)

By R. Kawashima and M. Nagata.

(Agr. Chem. Laboratory, Kyushu Imperial University; Aug. 17, 1939)

In this paper the morphological and some chemical natures of a saline alkali soil, strongly influenced by underground water, have been described. The place examined lies 65 km west of Harbin.

The amounts of soluble salts in an extract of soil-water ratio 1 : 20 are given in table I.

Table I. Soluble salts in milliequivalents per 100 g soil.

Layer	Thickness (cm)	Anions					Cations				
		SO_4	Cl	CO_3	HCO_3	Total	Ca	Mg	K	Na	Total
A_1	20	1.40	0.09	0.49	3.77	5.75	1.78	0.46	0.12	2.78	5.14
A_2	38	1.20	0.09	0.76	4.26	6.31	0.82	0.89	0.13	4.31	6.15
G_1	36	0.87	0.15	0.95	5.64	7.61	0.30	0.39	0.09	6.26	7.04
G_2	66	1.43	0.16	1.36	6.31	9.26	0.75	0.34	0.18	7.96	9.23

As in the above table the concentration of soluble salts in A-layer is much lower than that of G-layer and the per centage composition of divalent cations is much higher than that of G-layer.

Next, the pH-values and amounts of exchangeable cations are indicated in table II.

The base status of A_1-layer is typical of a calcium soil, and that of A_2-layer still preserves a good supply of divalent cations and shows no appreciable alkalinisation. Therefore, the soil conditions of A-layer are considered favourable. In

Table II. Exchangeable cations in milliequivalents per 100 g soil.

Layer	pH	Cations					Per cents of equivalents			
		Ca	Mg	K	Na	Totat	Ca	Mg	K	Na
A_1	8.36	28.25	0.65	0.26	0.58	29.74	95.0	2.2	0.9	1.9
A_2	8.75	19.33	6.66	0.14	1.81	27.94	69.2	23.8	0.5	6.5
G_1	9.19	10.81	8.17	0.25	5.91	25.14	43.0	32.5	1.0	23.5
G_2	9.37	7.03	6.26	0.22	7.35	20.86	33.7	30.0	1.1	35.2

spite of the deleterious character of G-layer, the reclamation of this soil will be successful.

On the Acid Mineral Soil in South-Manchuria.

(pp. 815～818)

By R. KAWASHIMA.

(Agr. Chem. Laboratory, Kyushu Imperial University; Aug. 14, 1939.)

An acid mineral soil of low lime saturation is widely distributed along the coastal region of South-Manchuria.

Owing to the influence of herbaceous covering the acidity of surface soil is generally weaker than that of the subsoil

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On the Application of Hydrogen Peroxide for Brewing.

(pp. 819～830)

Part VI. On the effect of hydrogen peroxide on growth or multiplication of micro-organisms.

By Hisao MATUI and Masakazu YAMADA.

(The Governmental Institute of Brewing, Takinogawa, Tokyo;
Received July 19, 1939.)

Hydrogen peroxide has the effect to prevent the propagation of micro-organisms. The present authors have studied the behaviour of hydrogen peroxide upon the micro-organisms of the brewing use.

The saké yeast and the distillery yeast or kōzi fungi (*Aspergillus oryzae*) was inoculated in kōzi-extract of 10° Bllg. containing 1/10000～3/1000 part of 35% hydrogen peroxide, then incubated at 27～30°C. In these conditions the saké yeast could not propagate itself in the medium containing 8/10000 part of 35% hydrogen peroxide (ca. 0.028% H_2O_2) and the distillery yeast failed to proliferate in the medium containing 7/10000 part of 35% hydrogen peroxide (ca. 0.024%

H_2O_2), while the kōzi fungi was so resistant to hydrogen peroxide, that it could not be prevented from germinating unless above 3/1000 part of 35% hydrogen peroxide (ca. 0.1% H_2O_2) was added to the medium.

Part VII. The complete prevention of putrefaction of saké by hydrogen peroxide.

By Masakazu YAMADA, Hisao MATUI, and Tokuitiro ŌHASI.

Salicylic acid is permitted to be added to the limit of 0.02% to saké as an antiseptic, but the putrefaction of saké can not be prevented completely with such a quantity. Especially when saké is diluted with water to make it congenial to the taste, it is more apt to putrefy. On account of this fact brewers in Japan have always suffered a serious loss. We succeeded in the complete prevention of the putrefaction of saké by the addition of hydrogen peroxide under the following conditions.

As we stated in a previous paper (see Part IV.), saké has catalase which destroys in a few hours one thousandth part of 35% hydrogen peroxide added to saké. And yet if a large quantity of hydrogen peroxide is added to destroy the catalase, the quality of saké shows a marked fall and becomes unsuitable to drink. Therefore we must first destroy the catalase in saké by the method we reported in Part IV.

Let about 4 mg of hydrogen peroxide be contained in 100 cc of saké, and saké putrefies no more even if it is diluted with water to 65%.

Part VIII. On the application of hydrogen peroxide for the preservation or the refining of beverages or brewages.

By Hisao MATUI and Masakazu YAMADA.

As we have previously mentioned (see Part VI and Part VII.), hydrogen peroxide prevents the propagation of micro-organisms and so it can be used as an antiseptic for saké. Moreover as it has the decolourizing and deodorizing power, there may be room for the application of it to beverages or brewages in general.

We investigated the influence of hydrogen peroxide upon the following various kinds of beverages or brewages.

1, When hydrogen peroxide is added to syōyu (soy-sauce), the syōyu foams at once and is soon decolourized extraordinarily, but gives out a bad smell simultaneously. The bad smell is derived from aldehydes which come from oxidation of amino acids in syōyu by hydrogen peroxide. On the other hand, hydrogen peroxide added to syōyu is gradually decomposed by catalase in syōyu or by mutual reaction with such substances as amino acids. Thus it was shown that hydrogen peroxide was quite inadequate to be used as rectifying agent or antiseptic for syōyu.

2. The amino acids mixture prepared from vegetable proteins and used as seasoning behaves like syōyu to hydrogen peroxide. Gradual decomposition of hydrogen peroxide occurs producing various aldehydes or bad smell.

3. When one thousandth part of 35% hydrogen peroxide is added to natural vinegar, the smell of bacteria or diacetyl is lost, and its quality comes to resemble artificial vinegar.

4. The addition of 1/1000~4/1000 part of 35% hydrogen peroxide can prolong the time of conservation of "Calpis", tomato-ketchup, syrup of coffee, soup, unsweetened evaporated milk, bean-curd (tōhu) or vermicelli.

Über Endoconidiophora Fimbriata (E. et H.) DAV.

(ss. 831~835)

Von Kenji MIYAJI.

(Landwirtschaftliche Hochschule zu Gifu; Eingegangen am 14. Aug. 1939.)

Über die Bestimmung des Glutathions in Geweben.

(ss. 836~840)

Von Akiji FUJITA.

(Aus dem Biochemischen Laboratorium des Kitasato-Instituts
in Tokyo; Eingegangen am 30. Aug. 1939.)

On the Ether Extract of the Bark of Cajuput-tree.

(pp. 841~842)

By Minoru ISII and Yasuyosi OSIMA.

(Department of Food Chemistry, Taihoku Imperial University, Taiwan;
Received Aug: 30, 1939.)

Cajuput-trees (*Melaleuca leucadendron* Linn.), the trunks of which are covered with a bark of soft spongy layers, are widely planted in Formosa.

From the ether extract of the bark, which amounted to twenty per cents of the material, we separated a new resinol melting at 304°C. It was composed of C 10.51%, H 78.42% and was confirmed as $C_{28}H_{45}O_3$. We named this new resinol "Melaleucin". By the acetylation of melaleucin, monoacetyl-melaleucin mp 280°C, $C_{30}H_{47}O_4$, was obtained.

On the Hydrolysis of Fats and Fatty Acid Esters. (I)

(pp. 843～856)

By Toyoki Ono.

(Chemical Laboratory of the Fish Meal Association of Japan;
Received Aug. 21, 1939.)

Studies on the hydrolysis of fats and esters have been made by many scientists, but have not yet given satisfactory results in regard to the mechanics of the enzymic hydrolysis on the substances above mentioned.

I attempted, therefore, to observe systematically the following subjects:

(1) Influence of temperature on the saponification of fats, oils, and esters.
(2) Influence of temperature on the enzymic hydrolysis of fats, oils, synthetic glycerides and esters.
(3) Examination of the intermediate products in the course of hydrolysis.
(4) Hydrolysis by means of organisms.
(5) Isomerism on hydrolysis.

I. Influence of Temperature on the Saponification of Fats and Oils.

Twenty-eight fats and oils were taken as samples from plant and animal sources, and the saponification was carried out in the homogenous medium consisting of benzol and alcoholic alkali solution.

Table I shows the "Rate of Reaction Velocity" at temperature between 30° and 10°C. K (reaction velocity coefficient) was calculated from the equation,

$$K = \frac{2.3025}{T_e t} \log \frac{T_t(T_0 - T_e)}{T_0(T_t - T_e)}$$

where, t…time (in minute) on saponification.

T_0…C. C. of HCl required for neutralising free alkali before the saponification begins.

T_e… 〃 〃 〃 〃 after the saponification was finished.

T_t… 〃 〃 〃 〃 after time "t".

Results

1. At lower temperature, the fish oils, especially whale oil, are attacked more strongely by alkali than the vegetable ones.
2. Such solid fats as cacao butter, cocoa nut oil, and butter fat are very rapidly hydrolysed at 30°C compared with oily fats.
3. At the beginning of saponification, the reaction velocity at 30°C is large through all fats, but between 40 and 180 minutes it is constant, and then after 3 hours it diminishes.

Table I. The Rate of Saponification Velocity ($K_{t+10}:K_t$)

	Whale Oil	Sardine Oil	Salmon Oil	Trout Oil	Shark Liver Oil	Cod Liver Oil	Alaska-Pollack Liver Oil	Spermaceti	Lard	Beef Tallow	Butter Fat	Chicken Fat
$K_{t_1}:2\times K_{t_2}$	2.33	2.38	2.10	2.82	2.20	2.06	2.39	2.65	2.71	2.56	3.27	2.45
$K_{t_2}:K_{t_3}$	2.48	2.27	2.36	2.70	2.85	2.19	2.80	2.01	2.21	2.91	2.70	2.67
$K_{t_3}:K_{t_4}$	1.64	2.33	2.13	—	—	—	—	—	3.23	—	—	2.24

	Perilla Oil	Tsubaki Oil	Linseed Oil	Peanut Oil	Olive Oil	Soya Bean Oil	Castor Oil	Cocoa-Nut Oil	Japan Wax (Crude)	Japan Wax (Refined)	Cacao Butter	Rape Oil
$K_{t_1}:2\times K_{t_2}$	2.48	3.08	2.33	2.54	2.46	2.85	2.85	3.21	1.98	2.23	2.45	2.33
$K_{t_2}:K_{t_3}$	2.67	2.40	2.55	2.59	2.90	2.75	2.70	3.51	2.27	2.32	3.43	—
$K_{t_3}:K_{t_4}$	3.20	—	—	—	—	—	2.05	3.13	—	—	5.00	—

K_{t_1}, K_{t_2}, K_{t_3} and K_{t_4} indicate the reaction velocity coefficient at 30°C, 10°C, 0°C and −10°C.

Chemical Studies on the Kikyo-root. (Report VIII)

On the Constitutional Formula of Platycodigenin (Kikyo-Sapogenin). (No. 1)

(pp. 857~861)

By M. TSUJIMOTO and R. SENJU.

(Agr. Chem. Laboratory, Kyushu Imp. Univ. and Agr. Chem Laboratory,
Kagoshima Imp. College of Agr and Forestry;
Received Aug. 10, 1939.)

SUMMARY.

(1) Crude platycodigenin. It is a light yellow crystalline powder which is accompanied with resinous and colouring matters.

(2) Purification. The following two processes were applied.

1. K-Salt of platycodigenin was separated in a pure crystalline form, then the salt was decomposed by HCl and free platycodigenin was obtained in a pure state.
2. The chromatographic adsorption analysis was applied. We made use of Al_2O_3 (adsorbent) and acetone (eluent) in this experiment. Platycodigenin was adsorbed in the upper layer.

(3) General properties. Colourless needle or prismatic Crystals. mp=242~243°C. Soluble in methanol, ethanol, glacial acetic acid, acetic anhydride and acetone; difficultly soluble in chloroform; insoluble in water, ether, benzen and petroleum ether. $[\alpha]_D^{31.7}=+59.45°$. Unsaturated monobasic acid. Liebermann's sterin reaction possitive.

Chemical Studies on the Kikyo-root. (Report IX)

On the Constitutional Formula of Platycodigenin (Kikyo-Sapogenin). (No. 2)

Determination of the Molecular Weight of Platycodigenin.

(pp. 862~864)

By M. Tsujimoto and R. Senju.

(Agr. Chem. Laboratory, Kyushu Imp. Univ. and Agr. Chem. Laboratory, Kagoshima Imp. College of Agr. and Forestry;
Received Aug. 21, 1939.)

Summary

(1) The molecular weight of platycodigenin was determined by the titration method, Barger's method and the analysis of K-Salt. The results were as follows:—

Titration method	516	Titration method (Micro-Pregle)	532
Barger's method	520	Analysis of K-Salt	540
Mean	527	Calc. for $C_{30}H_{48}O_7$	520

(2) The elementary analysis of platycodigenin was as follows:—

	C (%)	H (%)
Experiment	69.45	9.17
Calc. for $C_{30}H_{48}O_7$ (=520)	69.23	9.23

From the above results, it was concluded that platycodigenin has the formula $C_{30}H_{48}O_7$ and the molecular weight of 520.

Phosphoric Acid Absorbtion of Soils in Tyosen.

(pp. 865~870)

By Misu-Hideo.

(Agricultural Experiment Station, Government General of Tyosen;
Received Aug. 28. 1939.)

Über das Vorhandensein der β-Oxyglutaminsäure im Shoyu (Japanische Sojasauce).

(ss. 871~875)

Von Yukio Tomiyasu.

(Aus dem Agrikulturchem. Laboratorium der Kaiserl. Kyushu Universität, Fukuoka;
Eingegangen am 12. Aug. 1939.)

35 L Shoyu wurden unter vermindertem Druck auf etwa die Hälfte konzentriert, um es von dem reichlichen Eiweißkoagulum und vom Kochsalz zu befreien, der Rückstand wurde vorerst mit basischem Bleiacetat, dann mit Phosphorwolframsäure behandelt. Das Filtrat wurde mit Bariumhydroxyd neutralisiert, und der dabei sich bildende Niederschlag mit 1%iger Salzsäure nach der Vorschrift von Gulland und Morris (J. Chem. Soc., 1644, 1934) eluiert. Diese Behandlung wurde

dreimal wiederholt. Darauf wurde die Lösung im Vakuum zu Sirup eingeengt, und die darin enthaltenden Monoaminodicarboxylsäuren wurden als Bariumsalz nach Jones und Moeller (J. Biol. Chem., **79**, 429, 1928) in dessen alkoholischer Lösung ausgeschieden. Daraus wurde Glutaminsäure als salzsaures Salz abgetrennt, und dann das Filtrat zum Sirup eingeengt. Der Sirup wurde mit Chloramin T oxydiert und daraus ein dunkelrotes *p*-Nitrophenylosazon vom Schmelzpunkt 298°C gewonnen, das 22,05%igen Stickstoff enthielt. Eine Spur dieses Osazons zeigte charakteristische Blaufärbung in der alkoholischen Natronlauge. Diese Substanz stimmt also mit dem *p*-Nitrophenylosazon von Äpfelsäurehalbaldehyd überein. Somit ist der Nachweis geliefert, dass β-Oxyglutaminsäure im Shoyu vorhanden ist.

On the Enzymic Action of Nncleotid-like Substances.

(pp. 876~878)

By Tetutaro TADOKORO and Naomoto TAKASUGI.

(Hokkaido Imperial University, Received Sept. 4, 1939.)

The Chemical Investigation of the Oil of *Setaria italica* Beauv.

(pp. 879~884)

By Hannemon ITO.

(The Gifu Agricultural College; Received Aug. 30, 1939.)

The material employed in this investigation was produced in Gifu Prefecture in 1927 and 1935.

33 kilograms of ground material, on extraction by continuous percolation with hot ether, yielded one kilogram of oil.

Some constants of the natural oil were determined, with the following results:

	Glutinous	Common
Specific gravity (15°C)	0.9195	0.9193
Refractive index (30°C)	1.4710	1.4710
Acid value	51.95	41.94
Saponification value	164.03	159.60
Iodine value	131.82	105.07
Hehner value	93.40	94.45
Reichert-Meissl value	0 43	0.48
Polenske value	1.3	1 4
Acetyl value	33.14	31.37

The contents of unsaponifiable matters and solid and liquid fatty acids in the oil were determined with the following results:

	Unsaponifiable matters %	Solid fatty acids %	Liquid fatty acids
Glutinous	2.13	17.40	72.45
Common	2.39	15.05	70.03

Some constants of solid and liquid fatty acids were measured as follows:

Liquid fatty acids.

	Neutralisation value	Mean molecular weight	Iodine value	Refractive index (30°C)
Glutinous	203.76	275.37	121.48	1.4690
Common	203.41	275.84	98.64	1.4679

Solid fatty acids.

	Neutralisation value	Mean molecular weight	Iodine value	Melting point
Glutinous	214.27	261 85	25.98	22~26°
Common	210.09	267.06	22 77	22~26°

From the solid fatty acids, palmitic acid and carnaubic acid were isolated.

Liquid fatty acids were composed largely of oleic, linolic and isolinolic acids.

From the unsaponifiable matters, a phytosterol, melting at 139~40°C, with $[\alpha]_D^{20}=-22.1$, was isolated. Its acetate melted at 122~4°C with $[\alpha]_D^{20}=-27.7°$.

From the unsaponifiable matters, besides a phytosterol, squalene was obtained.

The Chemical Investigation of the Oil of *Phaseolus vulgaris* L.

(pp. 885~890)

By Hannemon Ito.

(The Gifu Agricultural College; Received Aug. 30, 1939.)

The material employed in this investigation was produced in Hokkaido in 1932, and was ground by means of a stone mill. 16 kilograms of the ground material (moisture 13.0 per cent.), on extraction by continuous percolation with hot ether, yielded 195 grams of oil.

Some constants of the natural oil were determined, with the following results:

Specific gravity (15 5°C)	0.9603	Iodine value	149.8
Refractive index (30°C)	1.4808	Reichert-Meissl value	1.03
Acid value	20.5	Polenske value	2.00
Saponification value	132.6		

The contents of unsaponifiable matters and solid and liquid fatty acids in the natural oil were determined with the following results:

Solid fatty acids 19.0 per cent, liquid fatty acids 63.3 per cent., unsaponifiable matters 7.0 per cent.

Some constants of solid and liquid fatty acids were measured as follows:

	Specific gravity. (12°C)	Refractive index. (30°C)	Neutralisation value.	Iodine value.
Liquid fatty acids	0.9322	1.4718	186.8	156.6
Solid fatty acids	—	—	199.6	33.9

Solid fatty acids were considered to consist chiefly of palmitic acid and carnaubic acid but the former greatly predominating. Liquid fatty acids were composed largely of oleic, linolic, and linolenic acids.

The unsaponifiable matters were treated with alcohol to divide roughly into two parts, one easily and the other difficultly soluble in alcohol. Each of them was recrystallised from alcohol several times and a substance melting at 137~8°C, with $[\alpha]_D^{17}=-33.6$ was obtained from the part easily soluble in alcohol, while from the difficultly soluble part a substance melting at 151~3°C, with $[\alpha]_D^{18}=-45$ was isolated. These substance were assumed to show the usual phytosterol colour reaction, but the analytical results proved that the contents of the carbon atom were about one per cent. less than the phytosterol. The emperical formula from their analytical data, however, was close to $C_{20}H_{36}O$, $C_{21}H_{38}O$ or $C_{21}H_{36}O$. The melting point and the specific rotatory power of the substance melting at 137~8°C were 132~3°C. and −45.4 at 22°C., and those of the substance melting at 151~3°C were 137~8°C. and −74.4 at 20°C.

Über die Enzyme des Reismalzes.

(ss. 891~902)

Von H. MUNAKATA.

(Aus der Agrikult. Chem. Labor., Kaiserl. Univ. zu Tokio;
Eingegangen am 7 Aug 1939)

Untersuchungen uber Vitamin C in Obstsaftfabrikaten I.

Einfluß des Unterschiedes der Herstellung und der Sterilisationsmethode auf den Vitamin C-Gehalt in Tomatensaft in Buchsen.

(ss. 903~914)

Von Choten INAGAKI.

(Lebensmittelchemisches Forschungsinstitut der Meiji Zuckerindustrie.
Eingegangen am 30 Aug 1939)

Es werden Untersuchungen ausgefuhrt uber den Vitamin C-Gehalt mit 5 Tomatensaftbuchsen von in- und auslandischen Waren.

Nach der titrimetrischen Methode mit 2,6 Dichlorophenol-Indophenol zeigt sich der Vitamin C Gehalt in allen auslandischen Waren 3 bis 5 mal so groß als der in inlandischen. Die Askorbinsaure im frischen Tomatenfleisch betragt 21,5 mg% und die in Schale und Samen 27,9 mg%.

Durch wiederholte Untersuchungen wurde die Existenz der Askorbinsaureoxidase in frischen Tomaten bestatigt.

Die Askorbinsaureoxidase in Tomaten wird durch 10 Minuten langes Erhitzen auf 65°C vollkommen zerstort.

Weiter wurde die Beziehung zwischen der Askorbinsaure und der Askorbinsaureoxidase untersucht, sowie die Herstellung von Tomatensaft in Buchsen reich an Vitamin C und die beste Sterilisationsmethode zur Aufbewahrung.

Vol. 15 October 1939 No. 10

Bulletin of the Agricultural Chemical Society of Japan.

ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

Forschungen über das Fleischeiweiß des Herings.

(ss. 915~920)

Von Saburô WAKAMATSU.
(Obama Präfekturfischereischule in Fukui; Eingegangen am 14, 9, 1939)

An dem Material von gefangenen Heringen in der Nahe von Otaru in Hokkaido forschte der Verfasser uber den Losungsgrad des Muskels mittels heterogener Losungsmittel, die Verteilung aller Arten von Eiweißkorpern, die Aminosauren des Fleischeiweißes, die Bestandteile des Fleischextraktes und die Purinbasen im Fleischeiweiß.

Studies on the Yeasts Found in "Miso".

(Supp. Contributions)

Part I. Morphological Properties.

(pp. 921~932)

By Masatoshi MOGI.
(The Brewing Laboratory of Noda Co. Ltd., Noda-machi, Chiba-ken, Japan;
Received Sept. 5, 1939)

Phosphoric Acid Absorbtion of Soils in Tyosen. II.

(pp. 933~938)

By MISU-Hideo.
(Agricultural Experiment Station, Government General of Tyosen;
Received Aug 28, 1939.)

Studies on Histaminase. I.

Chemical Method for Determining the Activity of Histaminase.

(pp. 939~946)

By Kiyooki TAKAHASHI and Toshio UMEDA.
(Laboratory of Osaka Factory of Sankyo Co., Ltd.; Received Sept. 13, 1939)

The activity of histaminase had been determined by animal experiments. A new chemical method is described which includes the preparation of an extract from hog kidney powder, adsorption of the enzyme on Japanese acid clay, inactivation of histamine solution by the adsorbate in 24 hours at 38°C and determination of the residual histamine.

Pauly's diazo reaction is used for the colorimetric determination and the value obtained is corrected by the amounts of histamine adsorbed on the acid clay.

Inactivation curve of histamine by the various amounts of histaminase is described.

On the Steppe Soil between Harbin and Tsitsikhar in Manchuria.

(pp. 947~952)

By R. KAWASHIMA.

(Agr. Chem. Laboratory, Kyushu Imp. University; Received Sept. 27, 1939.)

A study has been made of the physical properties and chemical composition of three soil profiles in the semihumid region between Harbin and Tsitsikhar. The results of analysis represent a fairly good similarity relating to both physical and chemical properties. An average percentage content of calcium in the exchangeable cations is 95.2. Therefore all of the soils belong to the absolute pedocal.

It is shown that the composition of the colloidal clay below 0.001 mm in diameter has an average composition expressed by the mean silica-sesquioxide ratio 2.74, silica-alumina ratio 3.6, and the composition of the colloidal clay in these soils is strikingly similar.

On the Hydrolysis of Fats and Fatty Acid Esters (2)

(pp. 953~965)

By Toyoki ONO.

(Chemical Laboratory of the Fish Meal Association of Japan;
Received Sept. 25, 1939.)

Saponification was carried out in 0.2 mol. alcoholic KOH solution which contains the same volume of benzene, and 0.2 g of fat in 100 cc.

I. Influence of amount of fat:

In case of containing less fat than KOH, the reaction velocity is constant independent of the fat-content, but it diminishes in case of a large quantity of fat.

II. Influence of concentration of alkali:

Table II shows the rate of saponification velocity in various concentrations of alkali.

Table II. Effect of Alkali Concentration.

	Sardine Oil					Cacao Butter
	40°C	30°C	13°C	5°C	80% alcohol	30°C
$k'':k'''$	1.504	1.867	1.192	1.099	1.253	1.790
$k':k''$	1.971	1.573	1.460	1.274	0.862	1.369
$k':k'''$	2.965	2.937	1.739	1.399	1.080	2.449

k', k'' and k''' indicate the reaction velocity coefficient in 0.2, 0.1 and 0.05 mol. KOH solution.

From the above results, the velocity in 0.2 mol. diminishes at low temperature: on the contrary in 0.05 mol. it increases.

III. Influence of solvent:

In case of alcoholic KOH solution not containing benzene, "k" of castor oil and perilla oil is greater than that of sardine oil, cacao butter or olive oil.

IV. Saponification velocity of esters:

Results are given in Table III, in which "k" represents the average value of the reaction velocity coefficient.

Table III. The Rate of Saponification of Triglycerides and Esters ($k \times 10^5$)

	30°C	1°C		30°C	1°C
Triglycerides:			Oleic acid iso-Butyl	145.93	11.79
Caprin	236.12	41.66	〃 Amyl	159.17	13.84
Palmitin	248.14	41.04	Lauric acid n-Propyl	224 00	—
Stearin	216.62	30.25	〃 iso-Propyl	107.90	—
Olein	223.67	35.56	Stearic acid n-Propyl	190.26	—
Erucin	215.15	27.25	〃 iso-Propyl	96.13	—
Ricinolein	160.29	28.18	Caprylic acid Ethyl	268.05	18.24
Esters:			Stearic acid Ethyl	208.34	13.68
Oleic acid Methyl	210.67	18.59	Ricinoleic acid Ethyl	213.37	19.75
〃 Ethyl	206.67	15.65	Phthalic acid Ethyl	477.10	27.25
〃 n-Propyl	194.21	16 78	Benzoic acid Ethyl	141.73	5 56
〃 iso-Propyl	121.85	11.96	Salicylic acid Ethyl	6.81	0.55
〃 n-Butyl	184.19	15.59	〃 Methyl	17.75	2.66

On the Cellulose Analysis and Bleaching Methods of Cellulose Materials.

(pp. 966~972)

By Sin-iti HONDA.

(The Institute of Chemical Reserch; Received Oct. 4, 1939.)

Part I. New Method of Cellulose Estimation by Bleaching Powder.

For the methods of plant cellulose estimation, S H. Jenkins and A. G. Norman had proposed to apply the chlorination in liquid phase in place of gaseous chlorine phase. In their new method *antiformin* (NaOCl) solution was used as the chlorination agent. Because in Nippon we could not gain such a strength of antiformin as they employed, but bleaching powder was obtainable easily, the present writer tried to substitute antiformin and 6% Na_2SO_3 with the extracted liqour of bleaching powder (contains about 10% available chlorine) and 4% Na_2SO_3.

The present writer used 4% Na_2SO_3 solution instead of 6% Na_2SO_4 solution for the following three reasons:—

(1) Na_2SO_3 solution can be used as the delignification agent even when so dilute as 1.5%.

(2) In the experiments of holo-cellulose, J. G. Ritter etc. extracted with 2% Na_2SO_3, and dissoved out loosely combined hemicellulose in holocellulose.

(3) While treatments with 3% Na_2SO_3 on direct fire is apt to bump, the treatment with 2% sulphite liqour boils gently and ligninchloride easily dissolves into sulphite solution.

Differences between the present writer's and Jenkins and Norman's procedures consist of the following three points:—

I. Throughout the present author's analysis, the preliminary treatment as well as every after treatment with the 2% sulphite liqour is refluxed 10 minutes.

II. While Jenkins and Norman had used 5 cc of antiformin (contains 15% available chlorine) in so-called neutral chlorination and 5 cc of antiformin (contains 3% available chlorine), the present writer used in every chlorination 5 cc of bleaching powder extract (contains about 10% available chlorine).

III. Although Jenkins and Norman had taken 5 minutes as one chlorination period in a dark place, in the writer's method all the chlorination periods were 10 minutes. The treatment in the dark was not a necessary condition.

To provide an example for his new method, the present author used Bakkoyanagi (*Salix Caprea L.*) and compared its cellulose content by three methods, that is, normal chlorine gas method, Jenkins and Norman's antiformin method and the present writer's bleaching powder method. These results are shown in Table I. In the table, the figures for the ordinary chlorine gas method gave lower contents both in total cellulose and α-cellulose, than those for the chlorination in liquid phase. The low value of total cellulose content by Jenkins' method might be ascribed to the more complete removal of hemicellulose by the alkalinity of

Table I. Comparison of analysis by various methods with bakkoyanagi (*Salix Caprea* L.).
(Oven dry state.)

Method of Chlorination	Gaseous state.	Liquid state (State of solution).	
Method of Analysis. / Components.	Modified procedure of Cross and Bevan's Method.	NaOCl-Method (Jenkins and Norman's).	Bleaching powder Method
Total cellulose (%)	-54.95	47.67±0 66	55.69±0 32
α-cellulose (%) (ashe-free)	37.27	39.88±0 16	39.26±0 41
In total cellulose;			
α-cellulose (%)	67.83	83 59±1 02	70.60±0.31
α-cellulose-ash (%)	—	0.36	0.09
β-cellulose (%) γ-cellulose (%)	32.17	16 05	28.45

antiformin liqour than attained by the present author's method. However, the α-cellulose content showed good agreement by both methods.

From these results the present writer's bleaching powder method may be recommend for exact cellulose analysis.

Biochemical Studies on the Sexual Organs of the Silk Worm, *Bombyx Mori* L.

Parts I and II. On the changes of some chemical constituents according to the development of the sexual glands (ovary and testis.)

(pp. 973～988)

By Takeo Nakasone.

(The Mie Sericultural Experiment Station; Received Sept. 20, 1939.)

The author investigated the changes of water, dry matter, nitrogen, glycogen and ash contents in the sexual glands (ovary and testis) according to the development of *Bombyx mori*, after the last instar (after the 4th moulting), and also the influence of both respiration injuries and the extirpation of one of a pair of glands.

The results obtained are summarized in the following tables.

Table I. China 17 Spring reared.

Time (days)	Total content in ten testes				Total content in ten ovaries			
	dry matter	crude ash	total nitrogen	glycogen (glucose)	dry matter	crude ash	total nitrogen	glycogen (glucose)
The 5th instar	mgr	mgr	mgr	mgr	mgr	mgr	mgr	mgr
1	2 40	0.025	0.745	0 109	0.81	0.08	0.808	?
2	2.75	0.09	0.851	—	1.06	0.08	0.942	?
3	3.10	0.11	0.908	0.118	1.18	0.09	0.970	0.74
4	4.10	0.115	0.982	—	1.35	0.105	0.998	+
5	4.50	0.12	1.209	0.152	1 49	0.156	1.133	?
6	6 25	0.14	1.305	0.428	1.60	0.158	1.113	0.36
7	6.35	0.25	1.447	0.188	2.25	0.160	1.137	1.90
9 cocooning	7 25	0.30	1.873	0.118	3.02	0.180	1.230	1.20
11 pupation	10.00	0.42	1.941	0.204	3.60	0.180	2.130	1.35
14	13.22	0.62	2.012	0.220	13d 5.40	0.210	4.28	?
20	12.15	0.60	2.355	0.132	16d 97.0	8.02	28.36	6.48
21 moth	9.00	0.52	2.080	—	534.0	10 22	49.63	47.62

Table II. Nippon 111 September reared.

Time (days)	Male; Body weight 10 heads	(Ten testis)		Water	Female; Body weight 10 heads	(Ten ovaries)		Water
		Fresh matter	Dry matter			Fresh matter	Dry matter	
The 5th instar	gr	mgr	mgr	%	gr	mgr	mgr	%
1	9.10	33.0	3.50	89.4	10.22	9.7	0.5	93.8
2	11.0	35.2	3.8	89.2	13.12	10.6	0.8	92.5
4	15.3	62.7	4.8	92.3	19.40	14.2	1.0	92.9
6	27.0	65.2	6.2	90.5	32.62	20.2	1.4	93.1
8 cocooning	34 8	91.2	8.5	90.7	37.40	27.0	2.0	92.6
13 pupation	13.12	146.2	15.4	89.5	17.43	54.0	6.0	89.2
16	12.27	176.2	20.0	88.6	17.02	111.3	17.0	84.7
20	12.02	177.3	19.4	89.0	17.80	2003.0	470.0	76.5
22	11.42	151.7	18.8	87.6	16.48	3283.0	745.0	77.3
25 moth	10.08	71.20	10.2	85.6	15.97	3255.0	785.0	74.6

There was little influence on the remaining gland, when one of a pair of glands was extirpated at the early period of the 5th stage of the silk worm larva. When the abdominal stigmata of one side of the silk worm larva were sealed by enamels the development of the testis and the ovary of the sealed stigma side became inferior to those of the open stigma side, but in case the sealing of stigma was carried out after pupation the influence was not evident.

Effects of Fertilizers, especially of Potassium, on Sweet Potatoes as Source of Alcohol. I.

(pp. 989~1005)

By Akira SUZUKI, Shinji DOI, Teijiro UYEMURA, Yinchang WANG,
Seiji TADA, and Rikizo TAKAHASHI.
(Agricultural Chemical Laboratory, Department of Agriculture,
Tokyo Imperial University; Received Oct. 7, 1939.)

On the Enzymic Action of Nucleotid-like Substances. (2)

(pp. 1006~1008)

By Tetsutarō TADOKORO.
(Hokkaido Imperial University; Received Sept. 16, 1939.)

Statistic Studies of Soils. (X).

(pp. 1009~1012)

By Dr. MISU-Hido and RIHOKI.
(Agricultural Experiment Station, Government General of Tyosen;
Received Sept. 25, 1939.)

On the Method of Quantitative Estimation of the Aroma of Scented Tea.

(A Preliminary Report)

(pp. 1013~1017)

By K. UENO.
(Tea Experiment Station, Shizuoka. Received Oct. 7, 1939.)

Vol. 15 November 1939 No. 11

Bulletin of the Agricultural Chemical Society of Japan.

ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

On the Physiological Difference between 1,3- and 2,3-Butylene Glycols.

(pp. 1019~1022)

By Kinichiro SAKAGUCHI and Osamu KANBAYASI.
(Agricultural Chemical Laboratory, Tokyo Imperial University; Received Oct. 21, 1939.)

(1) The synthetic media, which contain 1,3- and 2,3-butylene glycols, glycerin or aethylene glycol (each in 2%) as the sole sources of carbon, were inoculated with five species of moulds, five species of yeasts and ten species of bacteria. The rate of growth of the orgnisms and the formation of reducible matters in the media were then investigated.

(2) 2,3-butylene glycol was utilized almost in the same degree as glycerin, while 1,3-butylene glycol was not assimilated by all species except *Oidium lactis* and *Bacillus mesentericus.* It seemed to exert even an inhibitory effect upon the growth of some species in the above concentration.

(3) Ethylene glycol was utilized by *Bac. mesentericus* and all species of moulds tested, i. e. *Aspergillus oryzae* A, *Penicillium glaucum*, *Rhizopus chinensis*, *Monilia fructigena* and *Oidium lactis*.

Studies on the Yeasts Found on "Miso" (Supp. Contributions)

Part 2. Physiological properties.

(pp. 1023~1036)

By Masatoshi MOGI.
(The Brewing Laboratory of Noda Shoyu Co. Ltd., Noda-machi, Chiba-ken, Japan;
Received Sept. 5, 1939.)

Phosphoric Acid Absorption of Soils in Tyosen. III.

(pp. 1037~1041)

By MISU-Hideo.

(Agricultiral Experiment Station, Goverment General of Tyosen; Received Aug. 28, 1939.)

On the Fermentation Degumming of Waste Silk. Part V.

A Useful Thermophilic Bacteria for Fermentation Degumming.

(pp. 1042~1044)

By Hideo KATAGIRI and Toshio NAKAHAMA.

(Agr. Chemical Laboratory, Kyoto Imperial University and Kanebo Silk Factory; Received Oct. 19, 1939.)

Twelve strains of thermophilic bacteria were isolated from the degumming liquid under a high temperature.

An aerobic bacillus was found to be most effective for degumming of raw silk, when pure fermentations of the silk were carried out at 55°C with all the bacteria isolated.

The useful bacillus was concluded to reveal very much the same nature as *Bacillus robustus* Blau, and its degummase easily dissolved out into the cultural solution during 5 days' incubation, as was already ascertained with *Bacillus cereus sp.* (see Part IV).

On the Enzymic Action of Nucleotid-like Substances. (3)

(pp. 1045~1046)

By Tetutaro TADOKORO and Naomoto TAKASUGI.

(Hokkaido Imperial University; Received Sept. 30, 1939.)

The Influence of Monochromatic Lights on the Action of the Enzymes. [Report XXII~XXIII]

Especially on the Influence of the Same Intensity of Visible Absorbed Rays.

(pp. 1047~1051)

By Reitaro MURAKAMI.

(Agricultural College, Utunomiya; Received Oct. 10, 1939.)

An electric lamp was used in order to investigate the influence of the visible rays on the action of the saccharase and proteinase in the yeast.

The enzyme solutions were irradiated through the layer of copper sulphate solution and the monochromatic filters of red, green, blue and violet, and compensated to the same intensity of the absorbed rays. Colorless and black filters passing respectively all and no visible rays were also applied for controls.

The preparation of the enzymes, the measurement of the enzyme action and the other treatments were the same as in the author's previous papers.⁽¹⁾

In this experiment, the actions of the yeast saccharase and proteinase were promoted by the visible monochromatic lights. The promotion was found to be stronger under the rays of shorter wave length and to become gradually weaker with longer wave lengths.

(1) Bull. Agri. Chem. Soc. (Japan), **15**, 45 (1939); **15**, 78 (1939).

Researches on Bamboos in Taiwan as a Raw Material for Pulp. Part II.

Especially on the ashes of "α-cellulose" originated from
the pulps treated with 17.5% NaOH.

(pp. 1052~1056)

By Minoru Tutiya and Masao Imai.
(Industrial Reserch Institute of Taityu, Taiwan; Received Oct. 12, 1939.)

Using about four kinds of bamboos in Taiwan, the bleached pulps obtained

Table I

Quantity of Ashes and Percentage of its Components.

Components Ash. (%)			Ash	SiO_2	Fe_2O_3	SO_3	P_2O_5	CaO	MgO	MnO	K_2O	Na_2O	Total
Kei-tiku	Bamboo		1.77	12.20	4.86	1.70	1.62	1.78	0.97	1.94	12.70	56.30	94.07
	Bleached	Pulp	0.522	67.50	12.83	1.78	—	8.29	0.27	1.64	0.79	3.68	96.80
		α-Cellulose	0.213	50.92	29.57	4.92	—	1.64	—	0.50	1.64	5.33	94.51
	Un-bleached	Pulp	0.531	43.78	10.35	9 56	—	6.92	7.24	2.50	13.38	3.02	96.85
		α-Cellulose	0.457	55.27	10.90	7.27	—	9.34	3.75	1.25	1.38	3.73	92.89
Matiku	Bamboo		4.31	14.90	10.27	1.23	0.61	1.43	2.05	3.70	12.90	47.20	94.68
	Pulp		1.078	55.75	16.36	2.10	—	6.70	1.77	2.57	12.53	2.17	99.95
	α-Cellulose		0.258	19.07	34.53	4.55	—	18.42	4.16	5.72	6.64	0.82	93.91
Shitiku	Bamboo		2.54	8.15	7.00	4.33	0.50	5.99	1.91	4.34	16.00	47.50	95.72
	Pulp		0.158	10.88	12.95	2.59	—	43.17	2.02	1.73	16.70	2.90	92.94
	α-Cellulose		0.102	19.46	22.93	4.12	—	35.27	2.15	0.75	4.86	1.78	92.32
Mosotiku	Bamboo		1.18	14.10	8.60	2 30	2.50	2.80	1.40	3.70	27.50	34.00	96.90
	Pulp		0.823	19.59	18.58	7.52	—	13.52	5.52	6.19	18.12	5.28	94 30
	α-Cellulose		0.344	51.54	18.51	4.15	—	2.24	0.83	4.62	9.87	5.55	97.31
α-Cellulose of artifitial silk pulp	Ooji		0.156	43.72	10.77	2.92	-	4.87	—	—	2.47	30.56	95.31
	Raynier		0.023	37.25	27.45	3.88	—	9.80	—	—	9.80	5.88	94.06

Table II.

m. g. of the components to 100 gm of Bamboo and Percentage.

Ash. m.g. %				SiO_2	Fe_2O_3	SO_3	P_2O_5	CaO	MgO	MnO	K_2O	Na_2O	Total
Kei-tiku	Bamboo			215.94	86.02	30.09	28.63	31.50	17.16	34.33	224.79	996.51	1,665.01
	Bleached	Pulp.	m.g.	118.16	22.45	3.11	—	14.50	0.47	0.28	1.38	6.44	166.79
			%	54.71	26.09	10.33	0	46.03	2.73	0.81	0.61	0.64	10.00
		α-Cell.	m.g.	29.02	16.85	2.80	—	0.93	—	0.28	0.93	3.03	53.84
			%	13.43	19.59	9.30	0	2.95	0	0.81	0.41	0.30	3.23
	Unbleached	Pulp.	m.g.	83.61	19.76	18.25	—	13.21	6.22	4.77	25.54	5.75	187.11
			%	38.71	22.97	60.65	0	4.19	26.24	13.89	11.36	0.57	11.23
		α-Cell.	m.g.	69.64	13.73	9.16	—	11.76	4.73	1.57	1.73	4.69	117.01
			%	32.24	15.96	30.21	0	3.73	19.81	4.57	0.76	0.47	7.02
Ma-tiku	Bamboo			642.19	442.62	53.01	26.29	61.63	88.35	159.47	555.99	2,034.32	4,063.88
		Pulp.	m.g.	156.10	45.80	5.88	—	18.76	4.95	7.19	35.08	6.07	279.93
			%	24.30	10.34	11.09	0	30.43	5.60	4.50	6.30	0.29	6.88
		α-Cell.	m.g.	9.34	16.91	2.22	—	9.02	2.03	2.80	3.25	0.40	45.97
			%	1.45	3.82	4.18	0	14.63	2.29	1.75	0.58	0.01	1.13
Shi-tiku	Bemboo			207.01	177.80	109.98	12.70	152.14	48.51	110.23	406.64	6,206.50	2,431.51
		Pulp.	m.g.	3.80	4.53	0.90	—	15.10	0.70	0.60	5.84	1.01	32.48
			%	1.36	2.54	0.81	0	9.92	1.44	0.54	1.43	0.01	1.33
		α-Cell.	m.g.	3.67	4.35	0.78	—	6.70	0.40	0.14	0.92	0.33	17.29
			%	1.77	2.43	0.70	0	4.40	0.82	0.12	0.22	0.005	0.71
Moso-tiku	Bamboo			166.38	101.48	27.14	92.50	33.04	16.52	43.66	324.50	401.20	1,143.42
		Pulp	m.g.	99.53	37.53	15.19	—	27.31	11.50	12.50	36.60	10.64	250.80
			%	59.82	36.98	55.96	0	82.65	69.61	28.63	11.27	2.65	21.93
		α-Cell.	m.g.	29.37	10.55	2.36	—	1.27	0.47	2.63	5.62	3.16	55.43
			%	17.65	10.38	8.69	0	3.84	1.84	6.02	1.73	0.78	4.84

m. g. in Pulp 100 gm

	SiO_2	Fe_2O_3	SO_3	P_2O_5	CaO	MgO	MnO	K_2O	Na_2O	Total
Ooji-Pulp	60.76	14.96	4.05	—	6.76	—	—	3.43	42.47	132.43
Raynier	8.19	5.76	0.81	—	2.05	—	—	2.05	1.23	20.09

by the nitric acid process, and the "α-cellulose", originated from the pulps treated with 17.5% NaOH, the principal components of the ashes were determined quantitatively and analytically as above. (Table I, II).

1. The principal components of the ashes in the pulps and the "α-celluloses" are silicates and ferric (or ferrous) compounds. There is no general peculiarity of chemical behaviours in the ashes, some being more soluble and others not. The more soluble ones, on the whole, contain smaller quantities of ashes.
2. Phosphoric comounds in the original bamboos are entirely dissolved out in the process of pulp manufacturing.
3. The large quantities of potassium and sodium salts in the ashes of the original

bamboos, are almost dissolved away in the process, so that the existence of these compounds in the ashes of the original materials are not so troublesome for pulp making.

4. In estimation of the kinds of bamboos for pulp making, the most recommendable one is "Shitiku", followed by "matiku" "keitiku" and "mosotiku" in the order given.
5. Phosphates, potassium and sodium salts are most easily dissolved away, this suggests that those components probably exist in the same salts or in the same conditions in plants.

Biochemical Studies on the Sexual Organs of the Silk Worm, *Bombyx mori* L.

Part III. On the catalase of the sexual glands (ovary and testis).

(pp. 1057～1064)

By Takeo NAKASONE.

(Mie Sericultural Experiment Station; Received Sept. 20, 1939)

The author studied the catalase actions of the testes and the ovaries of *Bombyx mori* in various developmental stages, respiration injuries due to the enamel sealing of the stigmata, influence of extirpating one gland of a pair in a silk worm larva after the 4th moulting and finally some properties of the catalase in the sexual glands of the full grown larva. The results may be summarized as follows:

1. The optimum conditions for the catalase in the ovaries and the testes are about pH 6.60 in reaction and about 22°C in temperature, but the activities gradually become weaker after thirty minutes even under these conditions.

2. Activities of the catalase in the ovaries and the testes show two cycles according to the development of the silk worm after the 4th moulting, one in the larval stage and the other in the pupal stage.

3. There seems to be almost no changes in the catalase actions of the remaining gland, when one of a pair of glands were removed at the early period of the 5th larval stage.

4. The catalase action of the sexnal glands became very meak when the abdominal stigmata of one side of the silkworm were sealed with enamel in the larval stage, but there was no influence if sealed in the pupal stage.

5. The catalase actions of the mature eggs (with chorion) in the ovarian tubes are not recognized.

Studies on the Mucilage from *Rhodophyceae.* (III).

On Physical and Chemical Properties of the Cold and Hot Water Extract from *Chondrus ocellatus* Holmes.

(pp. 1065~1069)

By T. Mori and Y. Tutiya.

(Department of Agriculture, Tokyo Imperial University; Received Oct. 28, 1939.)

On a Carbohydrase Acting on the Mucilage from *Chondrus ocellatus* Holmes (I).

(pp. 1070~1074)

By T. Mori.

(Department of Agriculture, Tokyo Imperial University; Received Nov. 6, 1939.)

On the Production of 2, 3-Butylene Glycol by Fermentation. I.

(pp. 1075~1084)

By Kinichiro Sakaguchi, Kisetsu Ôhara and Sumihiko Kobayashi.

(Agricultural Chemical Laboratory, Tokyo Imperial University; Received Oct. 16, 1939.)

Ten strains of bacteria, which form 2,3-butylene glycol in the yields of 30~40% (the maximum being 46%) from glucose, were isolated from soils and vegetable matters. Seven of these strains were found to belong to *Aerobacter aerogenes* and its related species and the other three to *Aerobacter cloacae* and its associates. Besides the glycol some of them form remarkable quantities of ethylalcohol.

The optimal conditions for the glycol production have been studied; i. e. the temperature 37~38°C, the pH value of the medium 6~7, and the concentration of glucose near 8%. As the nitrogen sources, 2~5% of powdered soy bean cake, fish meal or rice bran with the addition of small quantities of anmonium sulfate are suitable for the industrial production.

The formation of 2, 3-butylene glycol may be directly detected in the fermenting culture media by the application of Voges-Proskauer's test·after the treatment of the media with dilute sulfuric acid and a solution of potassium permanganate.

On the Hydrolysis of Fats and Fatty Acid Esters. (III).

(pp. 1085~1096)

By Toyoki Ono.

(Chemical Laboratory of the Fish.Meal Association of Japan; Received Oct. 26, 1939.)

Hydrolysis of Fats and Oils by Pancreas and Ricinus Lipase.

(A) Method of Examination.

On pancreas lipase:

Weigh 0.2 g of the sample into a 20∼30 cc bottle, add 5 cc of distilled water, 0.5 cc of 2% $CaCl_2$ and 0.5 cc of 3% albumin, and emulsify the content by shaking for 5 minutes. Add 2 cc of Clark and Lubs' phosphate buffer solution and 2 cc of pancreas-glycerin extract, shake for 5 minutes, and hydrolyse at 40°, 20°, 0° and −7°C. After a certain length of time, free fatty acids are

Table IV. The Hydrolysis of Fats and Oils by Lipase.

I. Ricinus Lipase

Oil / t (min)	Olive oil	Tsubaki oil	Linseed oil	Perilla oil	Castor oil	Chicken fat	Sardine oil	Whale oil	Salmon oil
30°C	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
30	57.30	50.34	51.58	38.61	41.35	54.86	28.46	20.74	23.20
90	70.80	83.33	68.68	69.10	62.41	73.00	36.12	35.30	33.74
180	82.49	94 80	80.22	80.00	62.54	90.30	43.00	38.60	42.70
300	84.31	95.35	81.81	82.15	79.70	92.71	43.75	41.00	43.06
0°C									
120	7.30	3.82	7.56	—	5.34	—	5.34	3.70	6.00
300	—	—	—	20.14	27.07	4.90	24.65	—	—
1020	41.61	44.44	57.70	63.20	—	12.50	—	33.58	27.32
2400	—	50.00	72.46	83.33	74.74	17.30	61.43	73.73	—

II. Pancreas Lipase

Oil / t (min)	Olive oil	Japan wax	Linseed oil	Perilla oil	Cocoa-nut oil	Chicken Fat	Sardine oil	Whale oil	Salmon oil	Butter Fat
30°C	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
30	25.26	6.70	22.38	15.52	21.69	21.21	37.09	34.08	27.49	20.11
60	34.92	12.00	55.67	18.34	34.58	30.83	44.22	40.00	42.54	26.35
120	52.01	17.01	46.16	24.69	46.90	45.26	49.22	51.11	49.84	36.06
180	59.44	18.04	54.55	39.40	58.60	51.63	53.51	57.78	52.36	43.69
240	67.61	19.33	61.54	62.40	65.65	58.70	57.08	60.00	55.63	45.77
300	71.33	20.75	67.14	63.47	71.51	60.11	60.00	66.67	59.00	47.16
0°C										
60	13.37	6.70	10.91	8.46	7.97	8.42	15.41	16.15	8.51	—
180	19.76	6.78	12.59	10.86	11.02	9.19	24.55	25.48	11.78	11.23
300	21.25	6.82	17.48	11.45	12.66	9.95	28.23	31.11	18.98	19.28
480	23.03	6.88	20.84	17.50	14.07	11.03	30.53	34.37	23.69	—
900	26.75	7.48	24.48	21.16	19.00	12.25	31.67	41.48	28.01	24.69
1200	27.50	7.73	28.40	25.11	21.34	13.48	32.81	48.00	37.30	34.00
−7°C		Tsubaki oil				Cod liver oil				
120	4.90	12.17	—	13.41	—	9.40	12 55	8.15	10.73	10.40
300	5.65	22.91	—	22.00	—	17.00	14.27	15.11	12.44	11.10
1200	20.81	24 75	—	34.13	—	25.12	26.83	32.89	24.90	12.76
1500	23.48	25.71	—	35.54	—	29.74	27.28	34.08	37.96	17.66

in neutralized with $N/10$ KOH solution, adding 40 cc alcohol.

On ricinus lipase:

Weigh 0.4 g of the sample, add to it 2 cc of water, 2 cc of acetate buffer solution and 1 cc of castor-seed-water extrat, shake for 3 minutes, and then treat the same manner as above mentioned.

(B) Results.

The experimental results are given in Table IV.

(I) The pancreas lipase has more active hydrolytic power on fish oils, at lower temperatures, than the ricinus lipase, but vegetable oils are more hydrolysed at 30°C by the ricinus lipase than by the former.

(II) The action of ricinus lipase is extremely retarded by the higher unsaturated fatty acids produced during the hydrolysis fish oils.

Studies on Acetone-Butyl Alcohol Fermentation.

(II). Fermentation of Mono-Saccharides by *Bac. granulobacter pectinovorum* and *Bac. butanolo-acetoni.*

(pp. 1097~1110)

By Sigeyosi Horie.

(Agricultural Chemical Laboratory, Kyusyu Imperial University, Fukuoka;
Received Oct 23, 1939)

On the Cellulose Analysis and Bleaching Methods of Cellulose Materials.

Part II. Application of a New Method of Cellulose Estimation on Various Plant Analysis.

(pp. 1111~1117)

By Sin-iti Honda.

(The Institute of Chemical Reserch, Received Oct 4, 1939)

In order to test the value of the present author's new cellulose estimation method, several plant materials were analysed by this method. In Table I, it is seen that bleaching powder method gave higher figures for total cellulose as well as for α-cellulose content than the ordinary chlorine gas method as in the case given in Part I of this paper.

Moreover, the procedures of the new method are simpler and can be carried out in shorter time than the ordinary Cross and Bevan method. Therefore, we may recommend this bleaching powder method for the estimation of plant celluloses. The purity of total cellulose and α-cellulose obtained by this new method

Table I. Comparison of plant cellulose contents by different analitical methods with various materials.

(Oven dry state.)

Name of plants.	Analysis by bleaching powder					
	Total cellulose (%)	α-cellulose (ash-free) (%)	In total cellulose			
			α-cellulose (ash-free) (%)	α-cellulose ash (%)	β-cellulose (%)	γ-cellulose (%)
Hosobaisotutuji (*Ledum palustre* L. var. *vulgare* Ledeb.	42.21±0.36	30.30±0.82	71.80±2.55	1.00	27.20	
Goyo no matu (*Pinus koraiensis* Sieb. et Zucc.)	56.03±0.06	39.12±0.08	69 82±0.08	0.23	19.85	
Hitujigusa (*Poa glumaris* Trin.)	47 52±0.46	34.45±0.87	72.51±1.80	0.99	7.75	18.75
Rice straw (*Oryza sativa* L. Asahi.)	42.56±0.51	30.70±0.25	72.80±0.81	2.34	30.70	—
Himemizugoke (*Sphagnum Fimbritum*, Wils.)	40.41	23.83	58.99	0.00		
Tundra, Surface layer	30.75	—	46 93	—	—	
〃 deep layer	25 84	—	—	—	—	

Name of plants.	Usual chlorine gas method.					
	Total cellulose (%)	α-cellulose (ash-free) (%)	In total cellulose			
			α-cellulose (ash-free) (%)	α-cellulose ash (%)	β-cellulose (%)	γ-cellulose (%)
Hosobaisotutuji (*Ledum palustre* L. var. *vulgare* Ledeb.	36.78±0.60	25.44±0.67	69.67±0.74	0.70	29.62	
Goyo no matu (*Pinus koraiensis* Sieb. et Zucc.)	52.55±0.09	34.37±0.41	65 41±1.0	—	34.59	
Hitujigusa (*Poa glumaris* Trin.)	44 10±0.49	32.53±0.88	73.77±1.16	—	26.33	
Rice straw (*Oryza sativa* L. Asahi.)	40.75±0.22	30.21±0.18	73.31±0.84	1.15	30.21	—
Himemizugoke (*Sphagnum Fimbrrium*, Wils.)	20.84	8.83	34.08	—	65.92	
Tundra, Surface layer	30.67	—	49.78	—	—	—
〃 deep layer	17.43	—	—	—	—	—

will be discussed in a forthcoming paper.

(Forest Chemical Laboratory, Department of Agriculture,
KyotoImperial University.)

The Influence of Monochromatic Lights on the Action of Enzymes.

Report [XXIV～XXV.]

(pp. 1118～1124)

By Reitaro MURAKAMI.

(Agricultural College, Utunomiya; Received Oct. 10, 1939.)

A Nitra-lamp was used to investigate the influence of the visible monochromatic lights on the action of the saccharase and proteinase in the yeast.

The enzyme solutions were irradiated through the layer of copper sulphate solution and the monochromatic filters of red, green, blue and viole, teach possessing three different intensities. Colorless and black filters passing respectively all and no visible rays were also used for controls.

The preparation of the enzymes, the measurement of the enzyme action and the other treatments were the same as in the author's previous papers.(1)

In this experiment, the actions of the yeast saccharase and proteinase were promoted by the visible monochromatic lights. The promotion was found to be stronger with the relative intensities of the absorbed rays, but to show the inverse effect in comparison with an unit intensity of all visible lights. The effect of monochromatic lights on the actions of saccharase and proteinase was found to be approximately proportional with the wave number.

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(1) Bull. Agri. Chem. Soc. (Japan), **15**, 45, 79, (1939).

Vol. 15 December 1939 No. 12

Bulletin of the Agricultural Chemical Society of Japan.

ABSTRACTS
from
TRANSACTIONS published in JAPANESE

(Pages refer to the Japanese originals of this volume unless otherwise noticed)

On the Stimulant for Cane Sugar Formation in Plants. (IV and V)

(pp. 1125~1129)

By Tetutaro TADOKORO.

(Biochem. Lab., Faculty of Sci., Hokkaido Imp. Univ.;
Received Nov. 16, 1939.)

Phosphoric Acid Absorbtion of Soils in Tyosen. IV.

(pp. 1130~1134)

By MISU-Hideo.

(Agricultural Experiment Station, Government General of Tyosen;
Received Aug. 28, 1939.)

On the Optical Properties of the Fermentation Lactic Acids.

(pp. 1135~1142)

By Hideo KATAGIRI and Shosuke MURAKAMI.

(Agr. Chemical Laboratory, Kyoto Imperial University; Received Oct. 30, 1939.)

(Part VIII) The Optical Specificities of Phosphoglycerates Decomposed by Lactic Acid Bacteria.

When the maceration extract of dried beer yeast or the resting cells of various kinds of lactic acid bacteria were added to Na *dl*-2-phosphoglycerate or Na *dl*-3-phosphoglycerate solutions with toluene, and kept at 26~30° for 0.5~

2.5 hr., the yeast maceration extract and most of the bacterial cells were found to show complete optical specificities, and pyruvic acid was always found as the product of decomposition.

With the yeast maceration extract, *d*(+)-2- and *d*(−)-3-phosphoglycerates were decomposed, while Leuconstoc mesenteroides var. Sake (*l*-acid former) decomposed *d*(+)-2- and *l*(+)-3-phosphoglycerates and Lactobacillus Sake (*d*-acid former) attacked selectively *l*(−)-2- and *d*(−)-3-isomers: the optical antipodes of the phosphoglycerates decomposed by *l*-acid former.

No optical specificity was found with Lactobacillus plantarum (*dl*-acid former), since all the forms of these phosphoglycerates were decomposed by the bacterial cells.

Experiments were carried out with the optically active 2- and 3-phosphoglycerates prepared from the *dl*-compounds by the action of *l*- or *d*-acid former.

The selective decompositions revealed by the bacterial cells were more clearly verified, i. e. *l*(−)-2- or *d*(−)-3-phosphoglycerates and *l*(+)-3-phosphoglycerate were never decomposed by *l*- and *d*-acid formers respectively.

In contrast with the case of *d*-acid former mentioned above, *d*(+)-2-phosphoglycerate was selectively decomposed by muscle extract which would produce *d*-lactic acid, whereas exactly the same selective decomposition of 3-phosphoglycerate was observed by the two *d*-lactic acid formers.

Therefore Embden-Meyerhof's scheme of lactic acid fermentation based upon the experiments with muscle extract, would at least be necessary to correct the optical property of 2-phosphoglycerate in the case of the fermentation with lactic acid bacteria.

Racemic form of phosphoglycerate was always recovered from the solution in which *dl*-acid former had been added to any one of the optically active components of 2- or 3-phosphoglycerates, therefore it is suggested that racemiase could again catalyse racemization of 2- and 3-phosphoglycerates as was already observed by us with lactic acid.

(Part IX) The Action of Racemiase upon Phosphoglycerates.

The racemization of *l*-(−)-2-phosphoglyceric acid effected by the resting cells of Lactobacillus plantarum (*dl*-acid former) was found to be due to racemiase, since the racemization was never observed with the maceration extract of dried cells of the same *dl*-acid former in which racemiase had been completely destroyed.

Exactly the same selective decomposition of phosphoglycerates was observed with the maceration extract of the *dl*-acid former as was observed with a *l*-acid former (Leuconostoc) with which *d*(+)-2- and *l*(+)-3-phosphoglyceric acids were decomposed, while no optical specificity was found with resting cells of the same *dl*-acid former.

Therefore it was ascertained that the action of racemiase was not limited to lactic acids, since the racemization of 2- and 3-phosphoglycerates was effected by the enzyme.

On the Solonetz Soil in Manchuria.

(pp. 1143～1148)

By R. KAWASHIMA.

(Agr. Chem. Laboratory, Kyushu Imp. University; Received Nov. 16, 1939.)

A Solonetz type soil is distributed along the Heisai line in the alkali soil region in Central Manchuria. The soil profile now concerned, has a loose and friable A layer, and a very hard large columnar B layer, although not so sharply structured, and an extremely hardened C layer. The texture of A layer is loamy sand, and those of B and C layers are respectively sandy loam and loam. Therefore, the illuviation of clay particles from above is manifested.

The chemical properties of these three layers are summarised as follows:—

The pH value, water soluble ash and calcium carbonate content are given in Table I.

Table I. The pH value etc.

Layer	Thickness cm	pH Value	Water Soluble Ash %	$CaCO_3$ %
A	30	8.64	0.020	1.585
B	25	9.86	0 218	3.995
C		10 27	0.252	7.812

As in Table I, the pH value and water soluble ash content of A layer are low, but those of B and C layer are very high. All of the layers contain calcium carbonate.

The water soluble salts in the extract of soil-water ratio 1 : 5 are determined and given as milligram equivalent per 100 gram soil.

Table II. Water Soluble Cations and Anions.

Layer	Anions (mg. eq. per 100 g Soil)					Cations (mg. eq. per 100 g Soil)				
	SO_4	Cl	CO_3	HCO_3	Total	Ca	Mg	K	Na	Total
A	0.18	0.04	0.19	0.98	1.39	0.35	0.35	0.05	0.54	1.29
B	0.50	0.30	0.77	2.54	4.11	0.17	0.21	0.27	3.64	4.29
C	0.67	0.29	1.34	2.59	4.89	0.38	0.21	0.30	3.85	4.74

As in Table II, the concentration of soluble salts in A layer is much lower than in B and C layer. The salts in B and C layer are composed principally of carbonate and bicarbonate of sodium.

The exchangeable cations determined by Puri's method are given in Table

III as milligram equivalent per 100 gram soil.

Table III. Exchangeable Cations.

Layer	mg. eq. per 100 g Soil				Percentage of Total		
	Ca	Mg	K+Na	Total	Ca	Mg	K+Na
A	7.07	2.31	0.22	9.60	73.7	24 1	2.3
B	4.21	2.47	4 74	11.42	36 9	21.6	41.5
C	2.09	2.21	7 40	11 70	17.9	18.9	63.2

As in the above table the exchange complex in A layer is well saturated with divalent cations, but in B layer the proportion of monovalent cations is increased surprisingly, and still more in C layer.

On the Selection of Grape Varieties for Wine Making. Part II.

(pp. 1149~1157)

By Zembei Kawakami and Takeji Hasegawa.

(Iwanohara Vineyard; Received Nov. 13, 1939.)

The present study is the continuation of previous work reported by H. Kawakami and S. Matumiya.⁽¹⁾

The grapes used in the present experiments are known varieties and crosses of European and American origin besides new crosses obtained by Z. Kawakami im Iwanohara vineyard, Niigata Prefecture. They are as follows:—

a) Fifty three known varieties of foreign origin and two Japanese native varieties, i. e., Concord, Adirondack, Unknown spec. No. 1, Kenena, Niagara, Prentiss, Bailey, Jona, Lady Washington, Brighton, Excelsior, Telegraph, Janesville, Beacon, Vergennes, Amber Queen, Agawam, Krueger, Bertrand, R. W. Munson, Atoka, Herbert, Admirable, Early Concord, Luckyne, Eaton, Husmann, Universal, Ives, Sado native grape, Cynthiana, Hybrid Franc, Jack, Wyoming Red, Elvira, Kôsyû, Big Extra, Herbemont, Black Sarara, Pierce, Isabella, Norton, Early Purple, W. B. Munson, Mills, Governor Ross, Weisser Steinschiller, Noah, Fern Munson, Big Hope, Highland, Hermann, Great Cluster, Newman.

b) Ten Z. Kawakami's new crosses, i. e., Big Extra x Folle Noire, Beacon x Alicante, Big Extra x Folle Noire, No. 5833, No. 5788, No. 5778, Bailey x Zinfandel No. 1, No. 1102 M x R, No. 503.

The varieties which gave wines of rather high quality are as follows:— Adirondack, Unknown sp. No. 1, Bailey, Big Extra x Folle Noire No. 3, No. 5788, Beacon, Brighton, Krueger, Herbert, Admirable, Eaton, Husmann, Cynthiana, Black Sarara, No. 1102 M x R and Newman.

(1) H. Kawakami and S. Matumiya: This Journ., 14, 1437 (1938).

The Studies on Bios. Part II.

(pp. 1158∼1160)

By Nobusada Okoti.

(Agricultural Chemical Laboratory, Faculty of Agriculture,
Tokyo Imperial University; Received Nov. 11, 1939.)

The bios content in oryzanin extract, oryzanin filtrate, oryzanin precipitate, malt and egg yolk has been studied.

On the Hydrolysis of Fats and Fatty Acid Esters (4).

(pp. 1161∼1172)

By Toyoki Ono.

(Chemical Laboratory of the Fish Meal Association of Japan; Received Nov. 17, 1939.)

I. Influence of Free Fatty Acids on Lipase Action.

The action of ricinus lipase was inhibited by fatty acids in the following order —the higher unsaturated fatty acids from sardine oil, capric acid, ricinoleic acid, palmitic acid and oleic acid. Especially the higher unsaturated fatty acids attacked ricinus lipase most strongly, but there was no influence on pancreas lipase.

II. Influence of Oxidised Oil and Hydrogen Peroxide on Lipase Action.

The oxidised perilla oil prepared by heating through the current of air, "blown oil", was less hydrolysed by ricinus lipase and hydrogen peroxide stopped completely the activity of lipase. On the contrary, pancreas lipase was not at all influenced by these two substances.

From these facts, I assume that the oxidised oils being less hydrolysed by ricinus lipase may be due to the inhibition by hydrogen peroxide produced from the peroxide compounds in oxidised oil.

III. Influence of the Amount of Water on Hydrolysing Power.

The amount of water used on emulsifying oil has a great influence on the hydrolysis of fats and oils. The suitable condition to make constant the value of "K" (the reaction velocity coefficient) is to use 5∼8 cc. of water and 0.2 g of fat.

IV. Influence of Organic Solvents.

In 30% glycerin solution, the action of pancreas lipase was diminished to 50 per cent. Alcohol inhibited in concentrations higher than 10 per cent and acetone did not inhibit but seemed to activate in the concentration of 10 per cent.

On Alcoholic Fermentation of Acorn, Saccharified by Sulphuric Acid.

(pp. 1173∼1178)

By Seisaku Sugizaki.

(Agricultural Chemical Laboratory, Department of Agriculture,
Tokyo Imperial University; Received Nov. 2, 1939.)

Studies on the Components of the Bark of *Rhamnus japonica* (4).

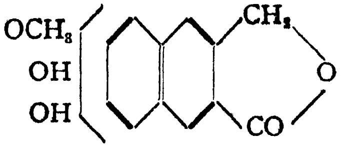
Determination of the Nucleus of α-Sorigenin.

(pp. 1179~1182)

By Ziro Nikuni and Hiroshi Hayashi.

(Agr. Chem. Laboratory, Tokyo Imp. Univ.; Received Nov. 27, 1939.)

In the previous report [Bull. Agr. Chem. Soc. of Japan: **15**, 43 (1939)], one of the authors deduced that the nucleus of α-sorigenin may be the lactone of 3-hydroxymethyl-β-naphthoic acid (or 3-hydroxymethyl-naphthalene-2-carboxylic acid).



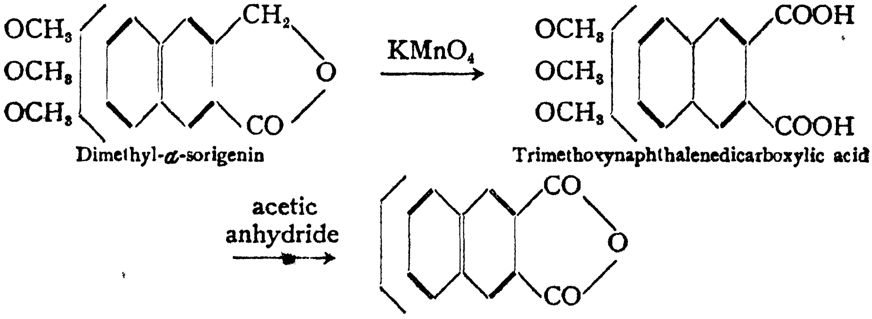
$=C_{13}H_{10}O_5$

α-Sorigenin

Now we confirmed that conclusion more decidedly from the following results.

1) On the oxidation of dimethyl-α-sorigenin with $KMnO_4$ in alkaline solution, trimethoxynaphthalenedicarboxylic acid is formed; yellowish plates melting at 258~261°, soluble in alcohol, hexane, 50% alcohol, alkaline solution and insoluble in hot water and acetic ether.

Boiling this dicarboxylic acid with acetic anhydride, the anhydride of this acid is formed as yellowish fine needles, m.p. 263~264°. Contrary to dicarboxylic acid, this anhydride is insoluble in alcohol and soluble in acetic ether.

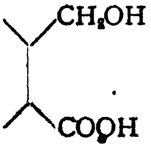


Dimethyl-α-sorigenin

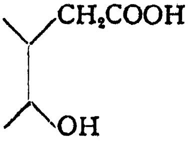
Trimethoxynaphthalenedicarboxylic acid

Anhydride of trimethoxynaphthalenedicarboxylic acid

From these facts the COOH groups must be in *o*-position to each other and α-sorigenin must be the lactone of the form A.



Form A;



Form B.

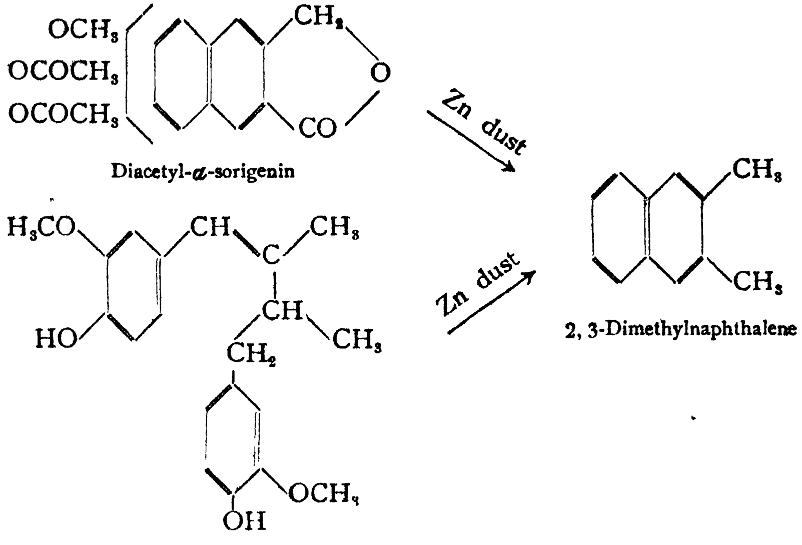
2) Comparison of the zinc dust distillation product from diacetyl-α-sorigenin with pure 2, 3-dimethylnaphthalene.

As reported before, the author obtained by the zinc dust distillation of diacetyl-α-sorigenin, fine colourless crystals with m.p. 90~96°, and confirmed it as 2, 3-dimethylnaphthalene from the results of analysis and absorption spectrum.

But the yield was too small to permit us to purify it completely to its real melting point (104°).

So we obtained pure 2, 3-dimethylnaphthalene by the zinc dust distillation of guajac resinous acid and compared its absorption spectrum with that of the above crystals.

The two spectra coincide very well on the main absorption maxima 288 mμ, 276 mμ, 266 mμ, and 253 mμ.



Diacetyl-α-sorigenin

Guajac resinous acid

2, 3-Dimethylnaphthalene

From the above results we decide that the nucleus of α-sorigenin is the lactone of 3-hydroxymethylnaphthalene-2-carboxylic acid.

The authors express their sincere thanks to Prof. B. Suzuki for his kind guidance throughout this work.

The Influence of Monochromatic Lights on the Action of Enzymes. [Report XXVI~XXIX]

Especially on the Influence of Ultraviolet Rays.

(pp. 1183~1192)

By Reitaro Murakami.

(Agricultural College, Utunomiya; Received Oct. 10, 1939.)

In order to further investigate the influence of ultraviolet rays on the enzymes in the yeast, the enzyme solutions containing saccharase, amylase, proteinase and lipase respectively were irradiated by ultraviolet rays from "Vim Ray" blue lamp. The treatments after the addition of the enzyme solutions into the substrates were the same as in the author's previous papers.(1)

In this experiment, the action of the yeast saccharase was found to be inhibited by ultraviolet rays and promoted by the visible rays. The saccharase was promoted at first under the influence of the rays containing both ultraviolet and visible, but the action of the enzyme was finally inhibited.

The amylase and proteinase were influenced very slightly by the action of lights. However, the action of the yeast amylase was found to be slightly inhibited by ultraviolet rays. The amylase was also inhibited at first by the visible rays and the rays containing both ultraviolet and visible, but the strength of enzyme was later regained. The action of the yeast proteinase was found to be promoted by the visible and ultraviolet rays and also promoted by the rays containing both visible and ultraviolet.

The action of the yeast lipase was found to be promoted by the visible and ultraviolet rays and also promoted by the rays containing both visible and ultraviolet.

(1) Bull. Agri. Chem. Soc. (Japan), **15**, 45~46; 79~80, (1939).

On the Alcohol-Manufacture from Iraqi Date-palm.

(pp. 1193~1200)

By Tadaaki Wada.

(Chemical Laboratory of Japan-China Oil Refining Co. Ltd.;
Received Nov. 6, 1939.)

Effects of Manuring on the Composition and Fermentation of Sweet Potato as a Source of Ethyl Alcohol.

(pp. 1201~1208)

By Seisaku Sugizaki.

(Agricultural Chemical Laboratory, Department of Agriculture,
Tokyo Imperial University; Received Nov. 4, 1939.)

Biochemical Studies on Bakanae Fungus of the Rice. Part IV.

The cultural condition for producing gibberellin or fusaric acid. I.

(pp. 1209~1220)

By T. Yabuta, Y. Sumiki, and S. Uno.

(Agr. Chem. Lab., Tokyo Imperial Univ.; Received Nov. 13, 1939.)

Studies on the Yeasts Found in "Miso" (Supp. Contributions.)

Part III. Classification.

(pp. 1221~1232)

By Masatoshi Mogi.

(The Brewing Laboratory of Noda Co. Ltd., Noda-Machi, Chiba-Ken, Japan;
Received Nov. 21, 1939.)